An Experimental Study of the Population Biology of an Ectoparasitic Digenean.

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AN EXPERIMENTAL STUDY OF THE POPULATION BIOLOGY OF AN ECTOPARASITIC DIGENEAN

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CHAPTER 1 INTRODUCTION

a) ABSTRACT

The survival and fecundity of the adult stage of the ectoparasitic digenean, Transversotrema patialense, on the fish host, Brachydanio rerio, have been experimentally examined by means of laboratory infections. The age and density dependence of these population parameters was investigated, and, in addition, the importance of the density independent parameter, water temperature, has been assessed.

The experimental results demonstrated that both survival and fecundity are age dependent and density dependent. Temperature was found to greatly influence both survival and fecundity, the optimum conditions being about 23°C. Fecundity was also found to be influenced by cyclical changes in illumination with most egg production occurring during darkness.

The survival and fecundity of flukes on reinfected hosts and the survival of flukes transplanted onto previously uninfected hosts provided no evidence for the existence of a host generated immune response to the adult fluke.

A niche parameter, host size, was examined and it was demonstrated that parasite survival was reduced on small hosts.

Growth of the adult parasite on the fish host was age dependent increasing rapidly in early life, rising to a plateau at approximately four weeks at 23°C., after which no further growth in size was observed. The vitelline glands exhibited a faster increase in area than the rest of the adult fluke in the first two weeks post infection. At high initial parasite densities, there was a density dependent reduction in parasite size.

The influence of different osmotic environments on the

infectivity and survival characteristics of cercariae, the survival of decaudated cercariae, and the survival and fecundity of adult flukes in vitro was investigated. Adult flukes became highly water sensitive within five minutes post infection, whereas decaudated cercariae did so to a much lesser extent. Survival of adult flukes in saline solutions was not enhanced by the addition of glucose or by the use of sterile conditions. Egg production was quickly terminated under in vitro conditions and was not affected by illumination.

These results are discussed in the context of existing literature on parasite population dynamics.

b) The Transversotrema life cycle as a laboratory biological model

The Transversotrema-Melanoides-Brachydanio system is an extremely useful biological model for the study of the biological and physical processes which control the population dynamics of complex host-helminth interactions. To gain insights into the population biology of such a system a detailed investigation of the many biological processes such as birth, death and transmission rates which govern the flow of parasites through their life cycles is ideally required.

This thesis is principally concerned with a detailed study of the adult parasite on its fish host. It is a component of a larger project which seeks to analyse the population dynamics of the whole life cycle of the parasite (fig.1). This type of approach to the study of host-parasite systems is extremely difficult to attempt in a field situation and has rarely been attempted in a rigorous manner within the laboratory. For example a task such as counting animal numbers, which is simple for many free living populations, is extremely difficult for endoparasites. To achieve comprehensive quantitative information on the processes outlined above, information which already exists for the population biology of some free living organisms and host-parasitoid systems, a laboratory biological model must be employed. The Transversotrema system has a number of characteristics which make it well suited to this role.

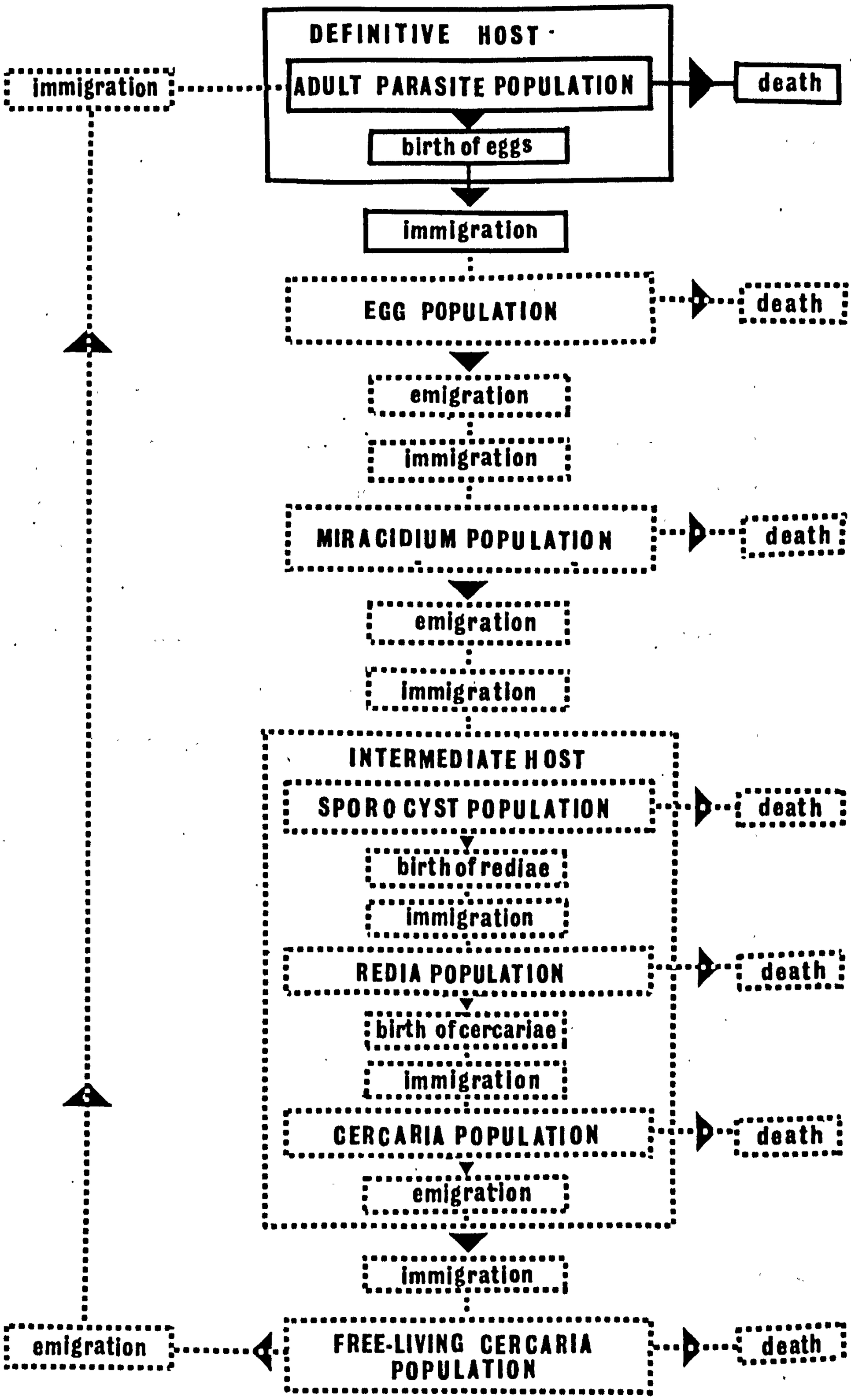
The definitive and intermediate host are both exceptionally robust and easily maintained under laboratory conditions. The definitive hosts are small, unlike those of some digeneans that have been used previously in laboratory experiments. Large numbers of these small fish hosts can be easily accomodated, enabling a high degree of replication where necessary. Compared with any mammalian host the maintenance costs of B.rerio are minimal. Both the fish and snail

Figure 1

Diagrammatic flow chart of the population processes involved in the life cycle Transversotrema patialense (Modified after Anderson and Whitfield 1975 and Anderson et al 1977).

n.b. There are at least two, and possibly more, distinct redial populations within the intermediate host. These are combined into one here for greater clarity.

The solid line demarcates those areas of the life cycle investigated principally in this thesis.


DEFINITIVE HOST
immigration
ADULT PARASITE POPULATION
death
birth of eggs
immigration
EGG POPULATION
death
emigration
immigration
MIRACIDIUM POPULATION
death
emigration
immigration
INTERMEDIATE HOST
SPOROCYST POPULATION
death
birth of rediae
immigration
REDIA POPULATION
death
birth of cercariae
immigration
CERCARIA POPULATION
death
emigration
immigration
emigration
FREE-LIVING CERCARIA POPULATION
death

hosts can be easily induced to reproduce in the laboratory and both occupy the same aquatic niche unlike some digenean life cycles that involve, for example, an aquatic snail and a largely terrestial vertebrate. Thus it is possible to maintain all stages of the life cycle in a single small heated aquarium for long periods with only minimal attention.

An outstanding advantage of the system is the ectoparasitic niche of the adult parasite, the adult parasite living under the scales of the fish host. This unusual site for an adult digenean enables the parasite population levels on the hosts to be assessed easily, accurately and non-destructively thoughout the duration of an infection. This is simply achieved by examining the surface of anaesthetized hosts under a low power microscope. The eggs pass directly from the parasite into the external aqueous environment. Thus no separation of eggs from faeces is necessary and a simple filtration technique enables the entire egg output of the parasites on a host to be easily assessed as often as required. This, in conjunction with the accurate determination of parasite numbers, enables mean egg output per parasite to be determined throughout the course of an infection on each host. In addition, it is extremely easy to manipulate parasite numbers by the removal of adult flukes at any stage in their development without apparent damage to the host. The adult fluke has a relatively brief life span on the definitive host and patency occurs extremely soon after infection.

Other parasitic stages in the system also present advantages for quantitive studies. For example, it is easy to observe and handle the cercaria due to its relatively large size. This enables infection experiments to be carried out with precisely known cercarial numbers and the production of parasite populations of predetermined size on individual hosts.

c) Transversotrematid biology

The family Transversotrematidae is a taxon of digeneans that are ectoparasitic on their definitive hosts. This ecto-parasitic niche is shared by only very few other digeneans, the avian eye fluke of the genus Philopthalmus being an example, (Fried, 1962).

The typical transversotrematid has a two host life cycle (fig. 2). Soparkar (1924) first obtained a cercaria from the snail, Melanoides tuberculatus (=M.tuberculata) in a stream serving irriga-tion ditches in the Punjab and named it Cercaria patialensis. The first adult transversotrematid, T. haasi, was obtained from an unknown Red Sea fish (Wittenberg, 1944). These and a number of other field observations all report transversotrematids infecting either snails belonging to the super-family Cerithiidae, or a wide range of fresh water, marine and euryhaline fish. A full record of the transverso-trematid species, their distribution and definitive and intermediate hosts is given in Appendix 1. These field observations have been augmented by the experimental work of Crusz, Ratnayake and Sathan-anthan (1964) and Sim (1972) who completed the life cycles of trans-versotrematid species in the laboratory. For both T.patialense and a Malayan variety of Transversotrema, the definitive hosts were fish, and the intermediate host, M.tuberculata (Appendix 1).

Adult transversotrematids inhabit recesses under the scales of their fish hosts (Crusz et al, 1964; Rao and Ganapati, 1967; Angel, 1969; Manter, 1965, 1970; Pande and Shukla, 1972; Sim, 1972). Velas-quez (1958) is alone in finding specimens of T.laruei in the intestine and muscle of the brackish water fish Lates calcifer and later (Velasquez, 1961) in the opercular cavities, gills and muscle. On this occasion, however, most specimens were located in the normal sub-scale site. Whilst a basically ectoparasitic organism could

FIG.2.

Fig. 2

A diagrammatic representation of the biological features of the life cycle of Transversotrema patialense. The parts of the diagram are not drawn to scale.

Key

a. Definitive host (Brachydanio rerio) showing a single adult fluke.

a1. An adult fluke.

a2. A section showing the adult fluke in the recess under the host's scale.

b. Mature, operculate, egg containing fully developed ciliated miracidium.

c. The intermediate host (Melanoides tuberculata).

d. The developmental stages of the parasite inside the snail host.

d1. Sporocyst (produces rediae).

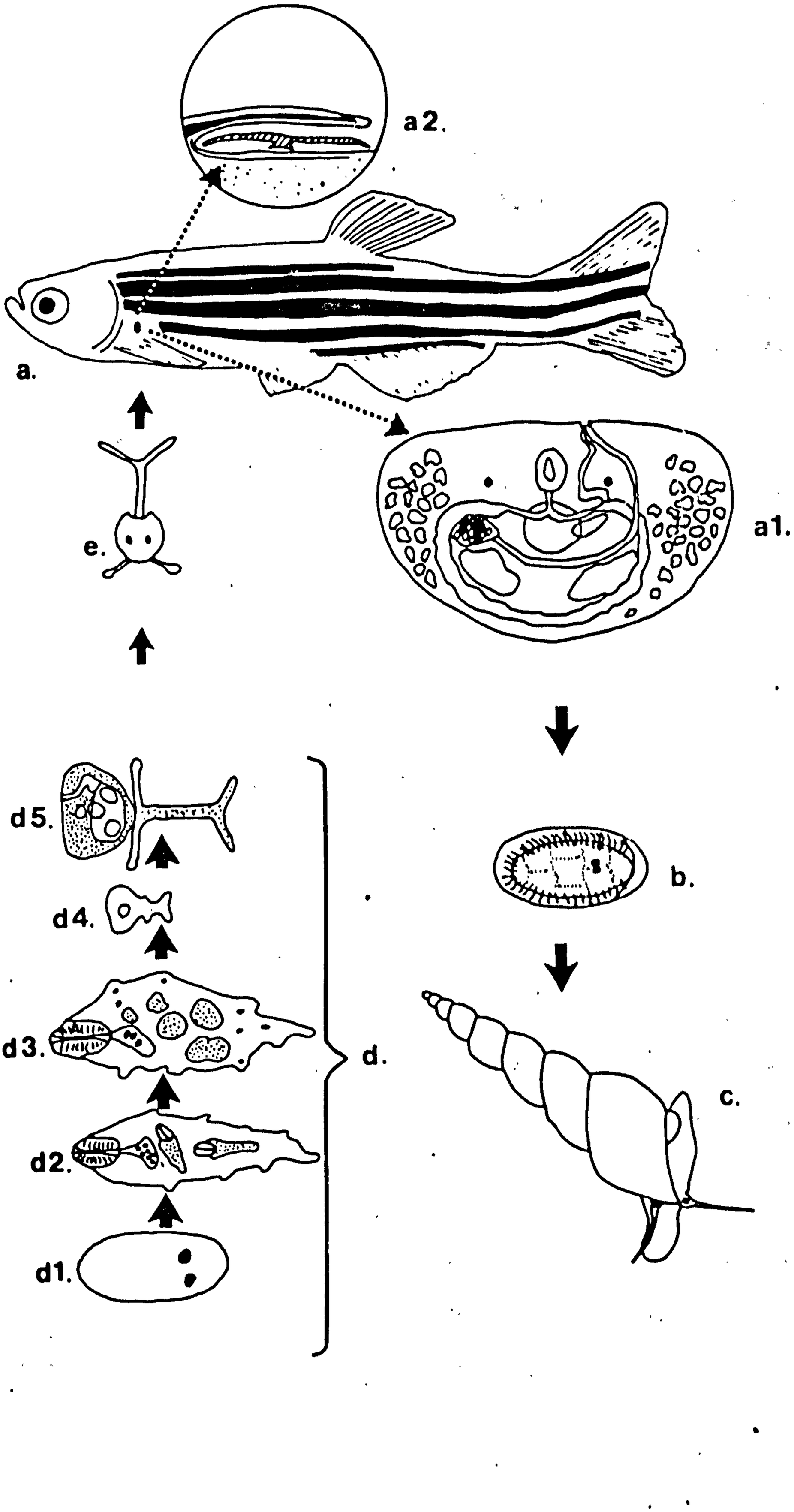
d2. Redia-producing redial generation.

d3. Cercaria-producing redial generation.

d4. Young cercaria.

d5. Mature cercaria.

e. Free swimming cercaria capable of infecting fish hosts.


a2.
a.
a1.
e.
b.
c.
d.
d5.
d4.
d3.
d2.
d1.

gain access to the gills and opercular cavity, it is hard to conceive mechanisms whereby it could also live in both the muscles and intestine. The small numbers of parasites reported from these latter sites (two from each) and the absence of such reports from morphologically similar species must cast doubts on the accuracy of these results; though only further work on this species (T.larvai)can resolve these anomalies.

The adult stage in the life cycle found under the fishes scales has been described as a progenetic metacercaria (Velasquez, 1961; Crusz and Sathananthan, 1960; Crusz et al, 1964) despite the presence of eggs in the uterus of some individuals. Olivier (1947) suggested that "the lack of evident adaptation for penetration and its precociousness suggest that the cercaria may develop directly into an adult in a fish or some other suitable host". Rao and Ganapati (1967) suggest that, despite the absence of eggs in the uteri of the specimens they found under the scales of fishes, these might, in fact, be adults and they considered that there might not be a metacercarial stage.

Later authors, whether or not eggs were present, have been in agreement that the stage living beneath fish scales was indeed the adult parasite (Manter, 1970; Pande and Shukla, 1972; Angel, 1969; Anderson and Whitfield, 1975).

From the uterus of the adult fluke tanned, negatively bouyant eggs are released directly into the water surrounding the fish host. Sim (1972) gives the only account of the egg's development for a "Malayan" species closely allied to T. patialense. She showed that a ciliated, bioccelate miracidium develops from a zygote within the egg after the egg's release. After 17-20 days the miracidium hatched from the operculate egg. Under Sim's experimental conditions the miracidium swam rapidly for 30 minutes after which, unless it

succeeded in infecting its intermediate host, M.tuberculata, its movements became increasingly sluggish before eventual death. The miracidium of this "Malayan" form was not observed to be strongly attracted to M.tuberculata and actual penetration of the snail was not observed.

The partial accounts of the developmental stages of transversotrematids in the intermediate host given by Soparkar (1924), Olivier (1947), Anantaraman (1948), Brien (1954), Velasques (1961), Pandy (1971), Nadakal, Mohandas and Sundararaman (1969) and Rao and Ganapati (1967) are all substantially in agreement except on some morphological features for the various transversotrematids described.

These morphometric characters are an unreliable method for contrasting soft bodied and highly contractile organisms. The following account of intra-molluscan development is based largely on that of Sim (1972) who has provided the most complete of the existing descriptions for the development of the "Malayan" form of Transversotrema.

Highly contractile and motile sporocysts were found close to the surface of the snail host, especially at the base of the foot. First generation rediae, produced by the sporocysts, migrated to the spaces between the snails digestive gland and the overlying connective tissue and haemolymph spaces between the digestive gland and the shell. The rediae have a prominent muscular pharynx (Soparkar,1924; Olivier, 1947) leading to an elongated and fairly narrow gut extending backwards nearly half way along the body (Sim, 1972). An inconspicuous birth pore is present about half way along the body (N. A. Moloney, Zoology Dept., King's College, London, personal communication). The shape of the first generation rediae varied from globular to pyriform (Soparkar, 1924).

In the first generation of rediae, germ balls developed

into daughter rediae. Cercarial production is commenced by the second or third redial generation (Sim, 1972).

Only partially developed transversotrematid cercariae are found within the rediae (Soparkar, 1924). After leaving the rediae they attain maturity in the digestive gland (Pandey, 1971). The time taken for the whole process from the infection of the snail to the first observed cercairial shedding appears to vary considerably from "well within" 35 days (Crusz et al, 1964) to 150-170 days (Sim, 1972).

All the transversotrematid cercariae described possess a similar unique morphological pattern. The body is highly flattened dorso-ventrally. The bifurcate tail bears two lateral arm-like processes at its proximal end. Anatomically the body of the cercaria is extremely similar to that of the adult parasite (fig. 3) The presence of motile sperm in the testes and seminal vesicles and of oocytes in the ovary suggest a degree of sexual precocity that is extremely unusual amongst digeneans. The only feature of the reproductive organs not showing a high degree of development is the vitelline system.

The swimming configuration of T.patialense has been described by Soparkar (1924) and its method of attachment to the definitive host by Rao and Ganapati (1967). The following account is based mainly on that of Whitfield, Anderson and Moloney (1975) who described these processes in greater detail. In its normal swimming configuration the cercarial head is hinged back and folded round the tail. It is probably anchored to the tail by its ventral sucker and the "arms" project anteriorly with respect to the cercarial head (Whitfield et al, 1975). This folding of the body is probably an adaptation to give a hydrodynamically efficient shape for the tail-first method of propulsion.

FIG.3.

Fig. 3

A diagrammatic representation of the main morphological features of a mature adult of Transversotrema patialense. A dashed circle marks the position of the ventral sucker.

e. pigment cup eye

g. genital opening

l. Laurer's canal

i. intestinal caecum

m. mouth

o. ovary

ot. ootype

p. pharynx

rv. right lateral vitelline duct

rt. right testis

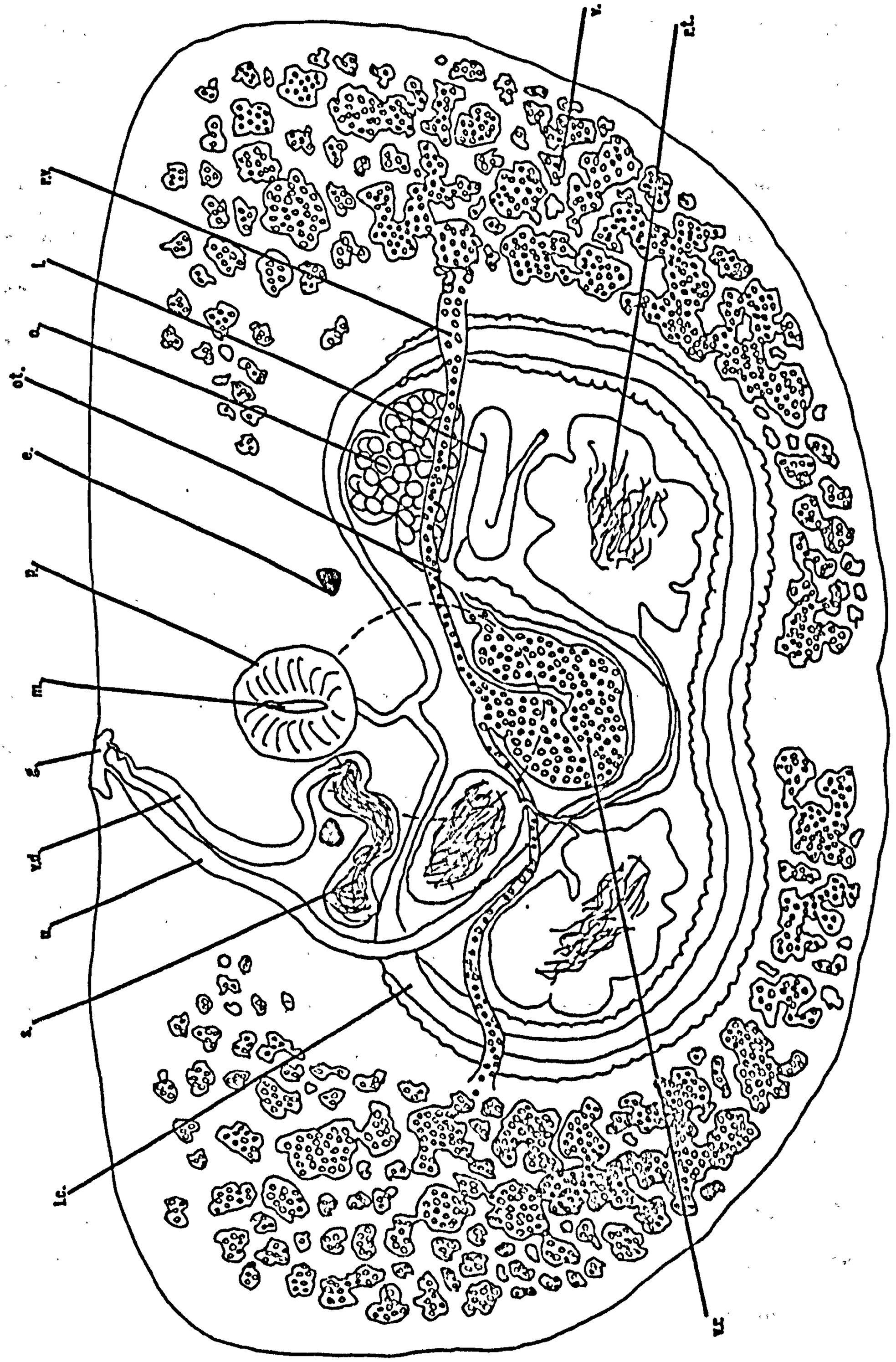
s. seminal vesicle containing motile sperm

u. uterus

v. vitelline cell

vd. vas deferens

vr. vitelline reservoir



The cercaria swims in an erratic path propelled by the beating of the tail stem and furcae. Brief bursts of swimming in a generally vertical direction are followed by periods of head-first sinking in which the tail furcae act to retard the sinking speed of the cercariae (Whitfield et al, 1975). As the finite food reserves of the cercariae decline, swimming activity progressively decreases, the instantaneous death rate of the cercariae increases and infectivity decreases (Anderson and Whitfield, 1975). Although there is no evidence that the cercaria is specifically attracted to the fish host the arm processes appear to function in both contact recognition and attachment to the fish host. The former role is mediated by mammiform receptors at the tips of the arms which appear to be contact chemoreceptors. At the distal end of each arm is an adhesive pad which is used to maintain contact with the host during attachment. The cytoplasm of the pad-region contains membrane-bounded adhesive granules, the contents of which are released during activation of the pad (Whitfield et al, 1975).

Once attached to a host by the pads the body of the cercaria hinges forwards from the tail bringing the dorsal side of the body into contact with the fish. One pad then releases its grip on the fish and the cercaria turns over to bring the ventral sucker into contact with the fishes surface. The cercaria crawls over the surface of the fish until the anterior edge of the body is pushed under the edge of one of the hosts scales. It then pushes its way under the scale after which the tail is shed.(Whitfield et al, 1975).

After two to seven days egg release commences (Sim, 1972). The shortness of the developmental period can probably be attributed to the progenesis displayed in the cercarial stage of transversotrematids. Only tail loss and the fuller development of the vitelline glands are necessary to convert the cercaria into a sexually competent adult.

The adult and cercarial body surfaces of transversotrematids are covered with backwardly directed spines arranged quincuncially. Their shape is variously described as,triangular (Soparkar, 1924) and shield shaped (Moloney, Zoology Dept., King's College, London unpublished data) for T.patialense, and shield shaped for T.haasi (Witenberg, 1944). These spines may help the adult flukes remain lodged beneath the hosts scales.

All the members of the genus Transversotrema have a morphology similar to that of T.patialense (fig.3). A point of controversy in the literature is the nature of the oral opening. Soparkar (1924), Olivier (1947) and Sim (1972) all consider the slit-like mouth to be surrounded by an oral sucker.and that the pharynx was absent. Witenberg (1944) described the mouth as leading to a globular pharynx and that there was no oral sucker. Velasques (1959, 1961), Anantaraman (1948) and Pandy (1971) dispute observations of an oral sucker and report the presence of a pharynx. There is, however, no dispute that only one structure is present in contrast to the one species described for the genus Prototransversotrema, P.steeri (Angel, 1969) which does possess both an oral sucker and a pharynx. From the description of Angel (1969) the oral structure present in the genus Transversotrema is not exactly analogous with either structure in Prototransversotrema.

d) The Biology of the Experimental Hosts.

i) Definitive Host: Brachydanio rerio (Hamilton-Buchanan 1822)

Although a wide range of tropical, and semi-tropical, fish are suitable definitive hosts for T.patialense (Whitfield and Wells, 1973), a small cyprinid, Brachydanio rerio, was selected for use in all the experimental infections carried out. The genus Brachydanio is native to India, Burma and Indonesia (Henovey and Hems, 1965); species in the genus are all omnivorous and oviparous. They are all ready for breeding by the age of one year and rarely live longer than three years. The genus contains one recorded natural host of T.patialense (Appendix 1 (Dr. C. Betterton, University of Penang, personal communication)).

B.rerio (common names Zebra danio, Zebra fish) is native to India from Bengal to the Coromandel coast. The back is olive green. Alternate strips of silver and blue extend from the gill cover to the tip of the caudal fin and are repeated on the anal fin. The dorsal and pelvic fins are hyaline. Hair-fine colourless barbels are present. In the wild a fork length of 50mm may be obtained though in the laboratory a length of 40mm was never exceeded.

B.rerio has been studied extensively by fish biologists because it is easily obtainable, inexpensive, resistant to disease, readily maintained and cared for, and, given appropriate conditions, will provide large numbers of eggs. B.rerio has been widely used in monitoring the effects of mutagenic, carcinogenic and teratogenic substances, as well as direct toxicants. An extensive review of the literature on B.rerio has been compiled by Laale (1977).

ii) Intermediate Host: Melanoides tuberculata (Muller).

Melanoides tuberculata (common name Malayan burrowing snail) has an extremely wide distribution ranging from South East and Southern Asia to Northern and Western Africa. Recently there have been reports of the establishment of M.tuberculata and related Thiarids in the Southern United States, Mexico and Puerto Rico, probably connected with their accidental release by aquarists (Clench, 1969; Murray, 1971; Abbott, 1972; Kotrla, 1975). M.tuberculata is common throughout Britain in the tanks of aquarists but could not become established ferally in our climate.

M.tuberculata is a member of the family Thiaridae, superfamily, Cerithaceae, and order Mesogastropoda (Wenz, 1938). The Thiarides are unusual in that they are parthenogenetic and viviparous. Few males have been found and the females brood the young in a pouch behind the head without any sexual process. Some members of the family harbour digenean parasites of medical importance including Opisthorchis viverrini and Paragonimus sp. (Ow-Yang and Yen,1975). M.tuberculata is a host for well over 30 digeneans (Sewell, 1922; Mohandas, 1974; Anantaraman, 1972).

The macroscopic anatomy of M.tuberculata has been described by Berry (1974) and in greater detail by Yousif (1975). The growth rate and reproductive anatomy have been described by Berry and Kadri (1974) who dissected over 400 specimens, all of which were female.

The external appearance of M.tuberculata is highly variable. The shell varies from prominently sculptured to smooth and from black to pale brown with a wide range of intermediate and patterned varieties. In some forms the tip of the spine tends to become eroded as the snails grow. Due to the parthenogenetic mode of reproduction natural cloning occurs and only one variety is usually found in any particular location.

PLATES 1, 2.

Plate 1

The experimental definitive host of Transversotrema patialense utilised throughout this study Brachydanio rerio.

Plate 2

The snail intermediate host of Transversotrema patialense, Melanoides tuberculata.

PLATES 3,4.

Plate 3

An adult specimen of Transversotrema patialense

(Courtesy P. J. Whitfield, Zoology Dept., King's College, London.).

Plate 4

A transverse section showing from the top downwards

1. A section through a scale of B.rerio
2. A section through an adult T.patialense lying below the scale
3. A section through another scale lying beneath the parasite
4. The epidermis and muscle of the host

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An adult specimen of Transversotrema patialense

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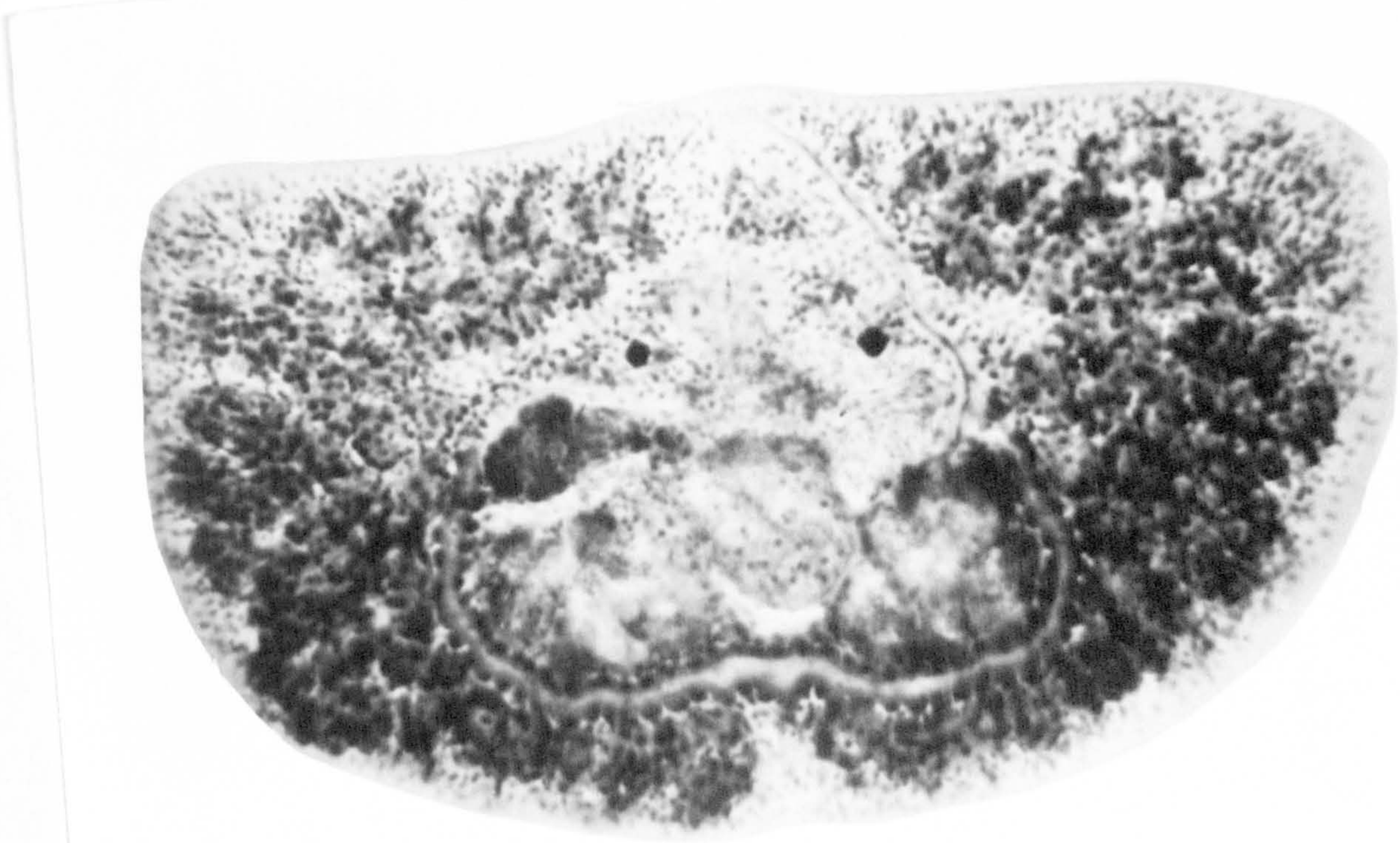
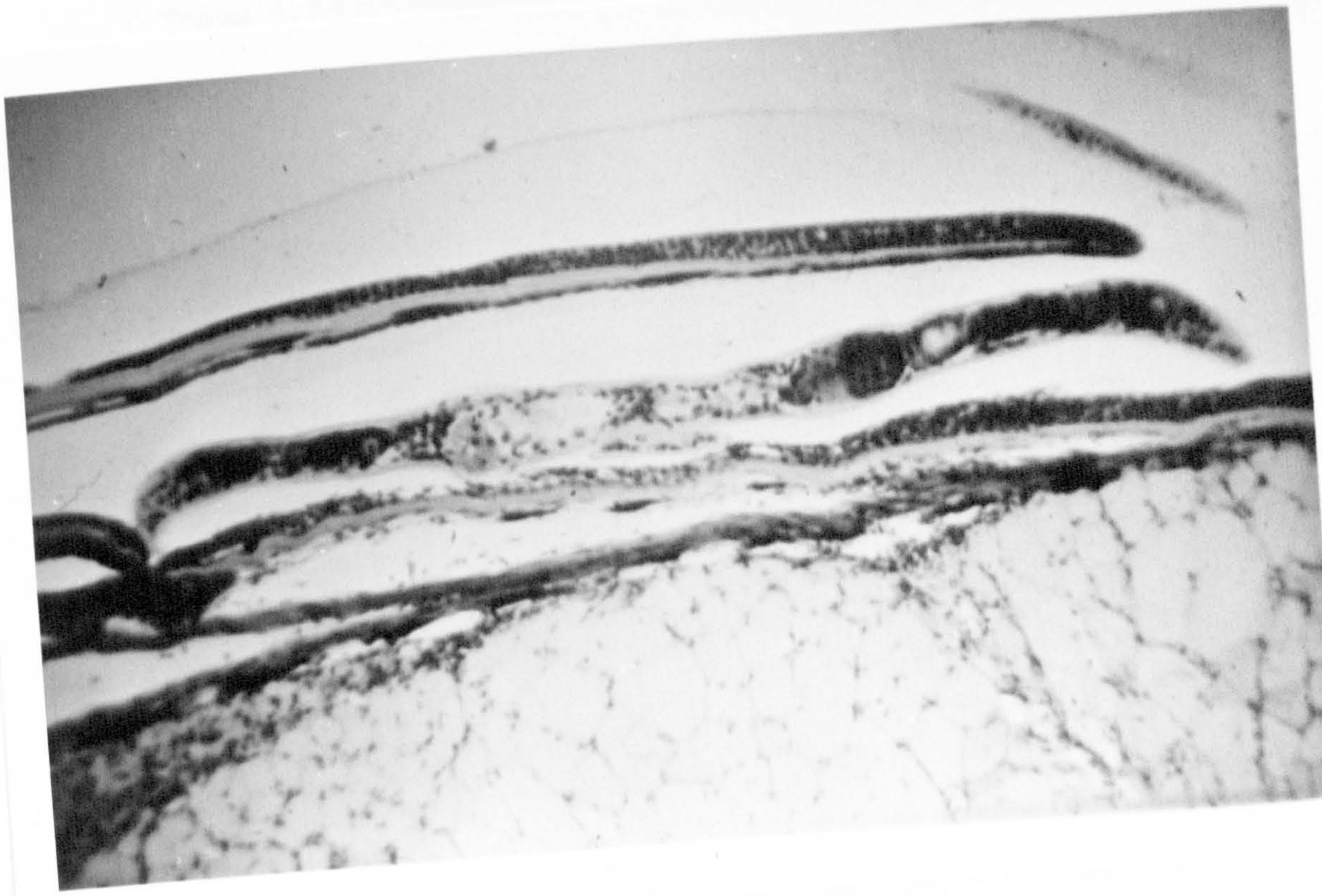




e) Parasite population dynamics

There is, in the ecological literature, a wealth of data on the population dynamics of free living organisms, obtained from both field and experimental work. The existence of this data has allowed the development of a wide range of mathematical models describing population processes. These models provide insights into the ways in which the interactions of free living organisms, with the physical, and biotic, factors in their environments, affect the dynamics of individual species, and multi-species interactions e.g. Southwood and Comins (1976) and Hassel, Lawton and May (1976).

Due to the ease with which the experimental manipulation of host-parasitoid interaction can be accomplished, a corresponding literature exists for the description of the dynamics of populations of parasitoid insects(Hassel (1968, 1971), Hassel and May (1973), Hassel, Lawton and Beddington (1976)).

These interactions however, have more in common, and indeed are a specialised form of, predator prey-interaction, because the host organism is invariably killed by infection with a single larval parasite.

The following review of the population dynamics of the true parasites is by no means comprehensive, but is intended to give a broad outline of current experimental, and theoretical, progress in determining those factors which influence the dynamics of parasite populations.

The growth and decay of parasite numbers are controlled by rates of change usually called population parameters, such as birth, death and transmission rates. The complex life cycles of parasite species may involve several distinct populations of parasites. This can include free living larval populations and stages, in one or more intermediate hosts, as well as the population in the definitive

host. This will increase the number of population, or rate, parameters controlling the dynamics of parasite populations.

These parameters constitute the components of a population interaction and hence dictate the population properties of two species interactions as between parasite and host. The population parameters are invariably functionally related to a variety of physical variables, such as temperature and many biological variables, such as the number of parasites per host.

There have been a number of experimental and field studies on the population dynamics of the free living stages of parasites. A common feature of these studies is the demonstration of age dependent survival, a pattern generated probably as a consequence of limited, and non-renewable, energy reserves. The larvae of many digenean parasites show age dependent survival, for example, the survival curves of the miracidium of Schistosomatium douthitti (Oliver and Short, 1956) and of Phyllodistomum sps. (Ubelaker and Olsen, 1970) and cercariae of Transversotrema patialense (Anderson and Whitfield, 1975; Anderson et al, 1977) and Schistosoma mansoni (Asch and Drane, 1972).

Examples among other groups include the first stage larvae of the nematode Bunostomum trignocephalum (Narain, 1965), the third stage larvae of Dictyocaulus filaria (Gallie and Nunns, 1976) and hexacanth larvae of Hymenolepis diminuta (Anderson and Lethbridge, 1975).

In addition, Anderson and Whitfield (1975) and Anderson and Lethbridge (1975), demonstrate a corresponding decline in the energy reserves of the cercariae and hexacanths respectively.

Survival can be influenced by a wide range of environmental variables such as temperature and humidity. The survival of free living larvae of Strongyloides ratti, for example, declines

above and below 15°C (Barrett, 1968) and survival of eggs and larvae of Haemonchus contortus decline above and below an optimum (Todd, Levine and Boatman, 1976). Survival of free living larvae of Dictyocaulus viviparus is affected by both temperature and humidity (Rose, 1956); survival of first stage larvae of Camallanus oxycephalus depends on salinity and temperature (Stromberg and Crites, 1974) and the survival of miracidia of Schistosomatium douthitti is dependent on temperature and pH.

Thus it would appear that the survival of these free living stages may be a complex function of age and environmental conditions.

The rate of transmission of an infective larval stage into a host population is governed by a wide range of processes. If the larval stage is free living, the age structure of the population may be of importance, due to reductions in activity and, hence, infectivity, with age. For example the rate of infection of copepods by Camallanus oxycephalus (Stromberg and Crites, 1974) and of fish by cercariae of Transversotrema patialense (Anderson and Whitfield, 1975) and Cercaria floridensis (Miller and McCoy, 1930) are all highly age dependent.

Apart from age dependence, infectivity is often influenced by environmental variables. Infection of Pisidium sp. by miracidia of Phyllodistomum sp. has been shown to be temperature dependent by Ubelaker and Olsen (1970a). Infection of mice by cercariae of S.mansoni is dependent on ionic status (Asch, 1975).

The size of the host environment appears to affect the immigration of the monogenean gill parasite Diplozoon paradoxum (Anderson, 1974) into its fish host. The age of the host affected infection by miracidia of Phyllodistomum sps.(Ubelaker and Olsen, 1970b). The prior history of parasite infections in the population can also be important. For example, a decreasing proportion of the

nematode cattle parasite Ostertagia ostertagi became established in hosts as the length of existing infections increased (Michel, 1973).

When a predator-prey relationship exists between definitive and intermediate host, the transmission rate may depend on the distribution of parasites in the intermediate hosts. For example, large populations of the eye fluke Diplostomum spathaceum, or the cestode Schistocephalus solidus, in Gasterosteus aculeatus, produce behavioural changes in the hosts which increase the chances of predation by the definitive host (Pennycuick, 1971). The feeding relationship between the intermediate and definitive hosts of Caryophyllaeus laticeps is governed by the density of the intermediate host in relation to alternative foods, the age structure of the host population and the season of the year (Anderson, 1974). The nutritional status of mice governs the rate of coprophagy and hence the rate of reinfection by the cestode Hymenolepis nana which can have a direct life cycle (Ghazal and Avery, 1976).

Parasite populations within, or on, a host may also exhibit age dependent survival. Two main causal factors appear to be operating to produce age dependence; one is senescence in the parasite, and the other is a time dependent immune response generated by the host. Often it is not possible to separate the influence of these effects because they occur concomitantly.

In the poultry cestode Raillietina cesticillus survival was markedly age dependent with little mortality in the first 42 days post infection, but few worms remaining after 112 days (Gray, 1972). In Nippostrongylus brasiliensis in the rat there is an initial loss of worms during migration through the host, followed by plateau phase in the intestine, and, finally, a decline in worm numbers. The form of this age dependence changes in subsequent infections when migratory loss increases, the plateau phase is shorter,

and the decline faster, probably due to increasing immune response by the host (Jarrett, Jarrett and Urquhart, 1968).

Fecundity may also be age dependent and often shows an initial increase which may be associated with parasite growth, a factor which may itself be age dependent. For example the growth of the eye fluke Philopthalmus sp. in ducklings (Fried, 1962); the growth of the acanthocephalan Polymorphus minutus in ducks (Crompton and Whitfield, 1968) and that of R.cesticillus (Gray, 1972) was age dependent with an initial fast growth rate which declines as size approaches a maximum. In the case of R.cesticillus, this is followed by a steep decline in mean size due to destrobilisation brought about by either an immune response, senescence, or both (Gray, 1972).

Age dependent reductions in fecundity may be associated, at the level of the host, with a reduction in worm numbers, or a reduction in egg output per individual parasite; a distinction which is often difficult to make accurately in the case of endoparasites. Again it is often impossible to distinguish the effects of senescence and immunity in declining fecundity.

In R.cesticillus proglottid production per host showed an initial steep rise, followed by a plateau and a decline which coincided with increasing parasite mortality (Gray, 1972). Hymenolepis diminuta infections in mice show a rise in egg output per mouse followed by a decline (Ghazal and Avery, 1974). Trichobilharzia ocellata in ducklings shows an age dependent decline in fecundity per host (Rau, Bourns and Ellis, 1975). This decline may be associated with the drop in worm survival between ten and twenty one days post infection (Bourns, Ellis and Rau, 1973). In Ostertagia ostertagi faecal egg counts, per calf, rise to a peak followed by a logarithmic decline (Michel, 1967).

Schistosoma mansoni infections of mice gave faecal egg

counts rising to a peak of 104 per worm pair 60 days post infection. Up to day 100 there was no apparent decline in worm survival, or in the rate of oviposition, indicating an increased retention of eggs in the host's tissue, possibly connected with a host generated immune response. There was, however, evidence of some worm loss between days 100 and 141 (Kloetzel, 1967).

Monkeys infected with S.mansoni show a dramatic fall in egg production three months post patency (Cheever and Powers, 1969). At the same time the number of worms recovered at autopsy falls and resistance develops (Smithers and Terry, 1965). The qualitative relation between the number of eggs in the faeces and parasite survival however, is ill defined, though Thompson, Meisenhelder, Moore and Waitz (1965) suggest that the overall decrease in egg production is due to decreased egg production by the remaining worms as well as worm death.

Moore and Sandground (1956) determined the daily rate of egg production per female S.mansoni and S.japonicum in hamsters for both passed, and retained, eggs for 21 days post patency. The autopsy of one animal, 83 days post patency, suggested that neither the rate of egg production per worm, nor the rate per host, showed age dependency.

Estimates for the rate of egg production by schistosomes vary widely, partly due to the high proportion of eggs retained in the tissue, and because, especially in human infections, it is difficult to obtain information on worm burdens. Estimates of mean daily egg output per female worm vary from 8-35,000 eggs (Macdonald, 1968).

If the host of a parasite is poikilothermic, environmental variables, like temperature, may affect survival and fecundity. For example, in the viviparous monogenean, Gyrodactylus alexandri, reproduction at 7°C is only one third the rate achieved at 15°C

(Lester and Adams, 1974). The tapeworm Caryophylaeus laticeps survived in the orfe for up to one month at 12°C, but for only three days at 18°C (Kennedy, 1971). Despite a failure to detect circulating antibodies against the worm (Kennedy and Walker, 1969), a temperature dependent immune response, rather than direct temperature dependent mortality, is postulated as the causal mechanism. Temperature was also the main factor controlling population levels of C.laticeps in the bream (Anderson, 1976a), although this author argued that its effects were direct and not mediated by an immune response.

Another class of mechanisms also operates on parasites within, or on, their hosts. These are density dependent factors and are generally considered to be of major importance in the long term stability of animal populations (Anderson, 1976b). These mechanisms may act to reduce parasite survival or fecundity or both. These regulatory processes may result from intra-specific competition for a limited resource in the parasites microenvironment, or a non-linear increase in the severity of host generated immune response with increasing parasite density.

Density dependent survival and fecundity have been observed in Hymenolepis diminuta with egg production per worm and per rat decreasing at population densities in excess of one worm per host (Hesselberg and Andresson,1975), and recoveries of worms were reduced at high infection levels (Andresson,Hindsbro and Hesselberg, in preparation). Similar results were obtained by Chappel and Pike (1976) with H.diminuta and Ghazal and Avery (1974) with Hymenolpis nana, except that in the latter case, the reduction in egg output per worm is at first offset by the increased number of individuals per host. A reduction in parasite size was also observed by Ghazal and Avery (1974) and Hesselberg and Andresson (1975) and earlier by Read (1951). This density dependent effect caused by a poor growth rate at high parasite densit-

ies generally results in a reduced rate of egg production per worm and possibly reduced survival. The term "crowding effect" was originally used in the description of these processes. Competition for limited resources, rather than an immune response by the host, is the most likely cause of density dependent growth as antibody levels were not found to be correlated with the number of H.diminuta present (Harris and Turton, 1973) and primary infections had little effect on secondary one (Roberts and Mong, 1968).

Similar results have been obtained from digeneans, especially flukes of the genus Fasciola. Density dependent growth (Ross, 1966), (Bitakarmine and Bwangamai, 1969), and densitydopendent egg production (Boray, 1969) have been observed in calves and Ross (1967) has reported density dependent survival.

Michel(1967) reported density dependent egg production in O.ostertagi with a similar egg output per host over a range of infection levels, so that there appears to be a limit imposed by the host microenvironment, limiting egg production to a fraction of the potential level, except at the lowest infection levels. Cortisone treatment raised, but did not remove the ceiling suggesting that both a competitive and immune component was involved. There was also a reduction in the proportion of mature worms (Michel, 1970) and density dependent growth (Michel, 1971); at high parasite densities density dependent survival was observed (Anderson and Michel, 1977). The varying nature of the immune response in the production of density dependent mortality has been well demonstrated in the case of Nippostrongylus brasiliensis in rats. A single very large dose of larvae results in a complete expulsion of worms, the so called "self cure" phenomenom (Mulligan, Urquhart, Jennings and Nielson, 1965). However, small, single or repeated doses, elicited no response and intermediate doses a partial one involving some density dependent mortality and a

decrease in egg production (Jenkins and Phillipson, 1970; Jarrett et al, 1968).

Another type of regulatory mechanism operates in the gastropod intermediate hosts of digenean parasites. The carrying capacity of the parasite microenvironment, and hence, cercarial output, is influenced by factors such as host nutritional status (Anderson et al, 1977) and the size of the host (Wright, 1971). Thus it is not surprising that the number of miracidia of Fasciola hepatica entering the snail Lymnaea trunculata has little effect on the rate of cercarial output (Wilson and Draskau, 1976), so the greater miracidial density, the more will tend to be "wasted" on infected hosts.

Increased host mortality, associated with increasing parasite burdens, can also act as a regulatory mechanism on parasite populations. The majority of parasite populations studied in detail exhibit overdispersion; for example, three endoparasites of Gasterosteus aculatus (Pennycuick, 1971a, 1971b) and populations of C.laticeps in Abramis bramae and Leuciscus leuciscus (Anderson, 1974; Kennedy, 1968). Crofton (1971a, 1971b) proposed a "lethal level" model based on the overdispersion of parasite populations. A consequence of the overdispersion of parasite populations is that a relatively small proportion of the host population will harbour a large proportion of the parasite population. Crofton (1971a, 1971b) proposed that those hosts in which the parasite burden reached the "lethal level" would die, thus removing a relatively large number of parasites from the population at the expense of only a few hosts. Anderson (1976c) states that the "lethal level" concept would still operate to regulate parasite populations however they were dispersed. Furthermore, Anderson and May (1977) provide empirical evidence to suggest that there is a gradual increase in host mortality with increasing parasite burden rather than an actual "lethal level". For example, Hayes, Bailer and Mitrovic (1973) found a positive correlation

between the number of metacercariae of F.hepatica administered to mice and the percentage host mortality, rather than a lethal level, and Grove and Warren (1976) obtained similar results with hamsters.

Perhaps the most characteristic feature of population studies on parasites in general, and helminth parasites in particular, is their scarcity. It is this paucity of imformation that has prompted the following study on Transversotrema patialense.

CHAPTER 2 METHODS

1. Transversotrema patialense life cycle maintenance

To ensure a constant supply of infected hosts for experiments the life cycle of T.patialense was maintained in the laboratory. The methods used were similar to those described previously (Anderson and Whitfield, 1975), (Anderson et al, 1977).

Standard polystyrene aquaria, capacity thirteen litres, were set up as life cycle tanks. Approximately three centimetres of fine gravel was placed on the bottom of each aquarium which was then filled with tap water. A growth of filamentous alga (Cladocera sp.) was established by the introduction of small quantities of this plant. The tanks were illuminated by 30 watt strip lights fitted to the undersides of the lids of the aquaria. The lights were controlled by a time clock (Smiths Autoset, Gallenkamp Ltd) to provide a 12 hours on, 12 hours off cycle of illumination and darkness. One hundred watt thermostat-heaters (Uno 100 watt automatic heater, Wholesale Tropicals Ltd) were placed in each tank and adjusted to give a water temperature of 23-25°C. The tanks were aerated continuously.

Between five and ten infected, and twenty and fifty uninfected, specimens of Melanoides tuberculata were placed together in each life cycle tank. They browsed on the Cladocera sp. and on other algae growing in the tank. At weekly intervals the snails diet was supplemented with between one and two dried boiled lettuce leaves.

The small cyprinid fish, Brachydanio rerio, has been shown to be a useful experimental definitive host for T.patialense (Whitfield and Wells, 1973) (Anderson and Whitfield, 1974). Between three and five fish of this species were kept in each life-cycle tank. They were fed five times a week on a proprietary fish food (Tetra-min, Wholesale Tropicals Ltd) in accordance with the manufacturers instructions. This food may also have formed part of the diet of M.tuberculata.

The infected snails in these life-cycle tanks shed cercariae which maintained the population of adult T.patialense on the fish. In turn the adult flukes under the fishes scales produced a constant supply of eggs. Inside these eggs miracidia developed and hatched to infect the uninfected snails in the population and reinfect those already infected.

Periodically the snails were checked for cercarial production (Methods 2) and some infected specimens were withdrawn from the life-cycle tanks to supply cercariae (Methods 3,4). In the conditions of the life-cycle tanks the snails reproduced parthenogenetically. At intervals juvenile snails produced in this way were removed with a net to prevent overcrowding.

2. Identification of infected snails

Periodically M.Tuberculata were removed from the life-cycle tanks and checked for infection. This was accomplished by placing each snail in a 20ml. specimen tube containing approximately 10mls. of tap water. The snails were then placed in the dark in an incubator (Illuminated cooled incubator with timed cycling IH-287, Gallenkamp Ltd) for one to three hours at 25°C. Darkness has been shown to stimulate cercarial release (Personal communication Drs. P.J.Whitfield and R.M.Anderson, Zoology Dept., King's College, London). The specimin tubes were then examined at low power (x8) against a dark background using a binocular microscope. The snails from any tube containing cercariae were transferred to infected snail holding tanks (Methods 3) and the others returned to the life-cycle maintenance tanks.

3. Maintenance of infected snail populations

Infected snails were maintained in conditions similar to

those described for the maintenance of the life-cycle (Methods 1). However, the tanks in which they were kept were illuminated continuously. The continuous illumination of infected snails tends to inhibit cercarial release (Personal communication Drs. P.J.Whitfield and R.M. Anderson, Zoology Dept., King's College, London).

4. Collection of cercariae from infected snails

To obtain cercariae for infection and other experiments, snails from the infected snail holding tanks (Methods 3) were placed in 600ml "Pyrex" crystallising dishes containing approximately 250mls of tap water. These were then placed in the dark in an incubator at 23-25°C. After half an hour the dishes were removed and the snails returned to the holding tank leaving cercariae available for use. In experiments where cercariae of a more precisley known age were required the periods of release were adjusted accordingly.

5. Counting cercariae

It was necessary to obtain known numbers of cercariae to infect fish. Cercariae obtained from the infected snail populations (Methods 4) were transferred individually from the 600ml crystallising dish into a 50ml crystallising dish using a Pasteur pipette and each transfer was recorded on a tally-counter. Only actively swimming cercariae were transferred.

6. Maintenance of fish stocks

A stock of B.rerio was maintained in conditions similar to those described for the maintenance of the life-cycle (Methods 1) except that M.tuberculata were rigorously excluded and the tanks subjected to the natural illumination cycle. The stock was periodically replenished with new fish (Wholesale Tropicals Ltd).

7. Maintenance of fish in survival and fecundity experiments

All infected fish were individually maintained in transparent rectangular polystyrene sandwich boxes with a capacity of 1500mls and filled with 1200mls of tap water. Each sandwich box had a lid in which a hole was drilled to enable a branch of an air supply system to enter. Through this system each tank was aerated for a twelve hour period each day.

In those experiments carried out at 23°C. a second hole was drilled in the tank lids to allow a 30 watt thermostat heater (Minimatic thermostat heater, Tachbrook Tropicals Ltd) to be fitted to each tank. These heaters were adjusted until the water temperature in each tank was 23°C.$\pm$ 0.5°C. These tanks were subjected to the natural light-dark regime.

In those experiments at other temperatures, the infected fish holding tanks and aeration system, was set up inside a cooled incubator (Pressed-Steel Fisher Ltd). This incubator was modified to give a 12 hours dark, 12 hours light cycle by means of a 60 watt bulb connected to an external time clock. Again the tanks were aerated continually.

In all cases the experimental fish were fed at least five times a week with a proprietary fish food. Periodically the fish were removed from the holding tanks and the number of flukes determined (Methods 11). Also, where necessary, the fish were not returned to the infected fish holding tanks. Instead they were placed in the egg collecting tanks to assess egg production (Methods 13). In all cases the egg collecting tanks were kept at the desired temperature in the cooled incubator. At the end of egg collection the number of flukes was again determined and the fish returned to the infected fish holding tank.

8. Fish breeding and maintenance of young fish

To obtain smaller fish for infection than those readily

available from suppliers, and to measure the growth rate of B.rerio, fish were bred in the laboratory. Several fish including a pregnant female, identified by its swollen abdomen, were placed in a tank containing Cladophora sp. and fine gravel. The tank was aerated and kept at 23°C. The female was examined daily and as soon as the abdomen appeared to be less swollen all the fish were removed to prevent predation on eggs and newly hatched fry. Three days later fry were observed clinging to the sides of the tank. For the following two weeks Liquifry fry food (Wholesale Tropicals Ltd) was added to the tank twice daily to supplement the abundant planktonic organisms present in the tank. For the next three weeks the fry were fed on fine powdered food(Fry Food, Wholesale Tropicals Ltd). After this they were fed the standard diet.

9. Measurement of fish growth

A group of fish was bred in the laboratory (Methods 8). Every seven days they were anaesthetised and measured. The distance between the tip of the mouth and the fork of the tail was recorded (fig. 6a).

For the first four weeks the anaesthetic, MS 222, was used at the reduced strength of 1:16,600 instead of 1:10,000 w/v to anaesthetise the fry.

10. Infecting fish

Before infection all fish were anaesthetised with MS 222 and examined to ensure no adult flukes were present (Methods 11). For the survival and fecundity experiments a series of parasite densities were required. To obtain infections at the level of one fluke per host, one cercaria was placed in a 50ml crystallising dish containing 30mls of tap water at 23°C. A fish was fed and then placed in the dish with the cercaria in an incubator for six hours. Under these

conditions over 40% of the fish became infected.

To obtain infections at densities of 2, 14 and 30 flukes per host, fish were placed in pots containing 6, 37 and 75 cercariae respectively under the conditions described above. This again produced infection rates of over 40%, resulting in infections slightly in excess of the desired levels in most fish. Any fish with fewer flukes than required were discarded. Those fish with too many flukes had the numbers reduced to the desired level (Methods 12). This process was completed within 24 hours of the commencement of the infection process.

To obtain larger infections fish were placed in pots with known numbers of cercariae. In this case the number of adult flukes was not subsequently adjusted to a predetermined level due to difficulties encountered in counting accurately the numbers of adult flukes present in large infections during the first 24 hours post infection.

For experiments where the number of adult flukes was not important fish were put in dishes with 75 cercariae using the method described above but the number of adults was not adjusted to a predetermined level.

11. Counting adult parasites in infection experiments

In infection experiments the number of adult flukes present on a fish was estimated directly by the following method. Fish were individually anaesthetised using a fresh solution of MS 222 (Sandoz) 1:10,000 w/v. This is a specific anaesthetic for cold blooded vertebrates. The anaesthesia was accomplished in Coplin Jar lids. Fish ceased to move actively and lay on their sides after approximately 60 seconds in the anaesthetic.

The fish were then examined for adult flukes using a binocular microsope at approximately x12 magnification. The two sides of

the fish were examined in turn for adult flukes. The dorsal and ventral surfaces were also examined as flukes were occasionally found here. The pectoral fins were pushed aside with a soft paintbrush to look for flukes hidden beneath scales under them. Adult flukes usually reveal their presence by their constant movement, which was made particularly evident because of the black eyespots. The flukes could be seen through the fishes transparent scales. Another useful initial indication of the presence of flukes, was the slight raising of scales under which parasites were lying.

12. Removal of adult flukes from fish

In order to obtain infections of the required size (Methods 10) and to provide adult flukes for *in vitro* culture and for transfers to other hosts, adult flukes were removed from the anaesthetised fish host using a microhook (fig. 5B).

The microhook consisted of a platinum wire loop-holder, fitted with a length of Hamilton Syringe cleaning wire measuring .08 mm in diameter at the tip. This wire projected 10 mm above the top of the loop-holder and the top 1.5 mm was bent round to form a hook.

The flukes were dislodged from beneath the scales using the tip of the microhook. Usually the dislodged fluke was left wrapped round the tip of the microhook. Occasionally, however, the fluke was dislodged into the anaesthetic from where it was removed with a Pasteur pipette to prevent reinfection. This operation was carried out against a dark background in a Coplin Jar lid at x12 magnification.

13. Egg collection technique

To collect the eggs which pass directly from the uterus of a mature adult fluke under the fishes scale into the water, a modified

floating breeding trap was used (Hykro 3 in 1 floating breeding trays, Tachbrook Tropicals Ltd). The breeding trap consists of a rectangular plastic tank with a capacity of 600mls, a lid and a false bottom; V-shaped in cross-section, with a slot at the base of the V. This insert rests about two-thirds of the way down the tank, dividing it into two chambers of unequal size, connected by the slot (fig. 4).

The traps were used as self-contained egg collecting tanks. To measure the egg output of the parasites on a fish, the latter was placed in 500mls of tap water in the upper portion of the egg-collecting tank. The tank was then placed in an incubator at the desired temperature and subjected to a 12 hours light, 12 hours dark cycle for 24 hours by means of a light inside the incubator connected to a time clock. Eggs produced by the parasites passed into the upper chamber of the tank and sank through the slot into the lower one thus preventing their ingestion by the fish. At the end of the 24 hour collection period the fish was removed. The contents of the tank, that is, water, fish faeces and eggs, were tipped into a beaker. About 100mls of tap water was then placed in the tank and shaken to dislodge any eggs remaining in the tank and this was then added to the bulk in the beaker.

The contents of the beaker were then filtered using a Millipore XX10-250 filter funnel connected to a water operated vacuum pump and Whatman 2.5 cm GF/A glass fibre filter papers. The beaker was rinsed with a little tap water and this also was passed through the filter. The filter paper was then removed and examined using a binocular microscope at about x16 magnification. The brown tanned eggs were easily seen on the white filter paper and were counted by pulling each egg off the filter paper using the microhook (Methods 12). Each withdrawal was recorded on a tally counter. Eggs adhering to the sides of fish faeces were included in the total. Eggs were occasionally visible inside the translucent faeces but were not recorded

because they were almost certainly ingested in the infected fish holding tanks (Methods 7). As a control an infected fish was kept for 14 days in an egg collecting tank and its contents regularly sampled using the method described above. Despite careful examination of the faeces they were not found to contain any eggs.

The accuracy of the egg collecting technique was determined by placing known numbers of eggs in egg traps, leaving the traps to stand with an uninfected fish for 24 hours and then determing the number of eggs present using the method described above.

FIG.4.

Legend for figure 4

Apparatus for collecting the eggs of T.patialense.

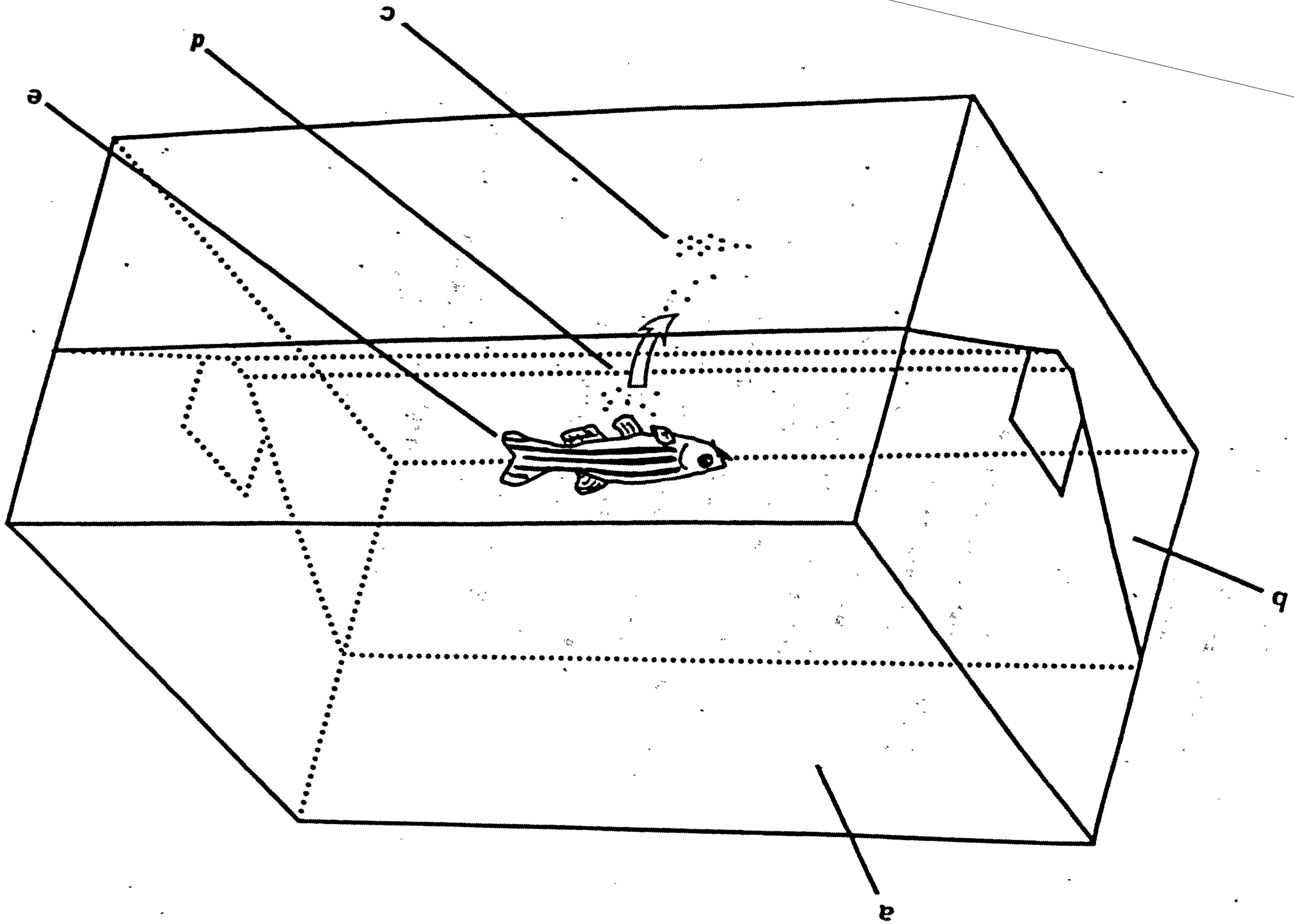
a. upper chamber containing infected fish

b. lower chamber

c. eggs of T.patialense

d. slot in false bottom through which eggs fall

e. infected B.rerio


c
d
e
a
b

N	Initial Number	Number Counted
1	50	48
2	50	50
3	50	50
4	50	48
5	50	50
6	50	50
7	50	49
	350	345

98.57% $\pm$ 1.76 (95% confidence limits) of the eggs were recovered.

14. Scanning electron microscopy

For the examination of the surface of infected and uninfected B.rerio scanning electron microscopical techniques were employed.

Specimens were fixed in 2.5% cacodylate buffered gluteraldehyde followed by dehydration in acetone. They were then dried in a Polaron critical point drier with CO_2 as the transitional fluid. Specimens were subsequently attached to the stubs with silver DAG (silver conducting paint) and sputter coated in an E500 Polaron diode sputtering system to an approximate thickness of 40 nm of gold/palladium before being examined using a Cambridge S4-10 scanning electron microscope at 20 KV.

15. Salt solutions

The saline solutions used most frequently, Contland Salt Solution and Hank's Salt Solution, have both been used in the cultivation of freshwater teleost cell lines and their compositions, along with that of frog ringer solution, have been described by Wolf (1963).

Ingredients (gms)	Cortland Salt Solution	Hank's Salt Solution	Frog ringer
NaCl	7.25	8.00	6.5
$CaCl_2.2H_2O$	.23	.19	.16
K Cl	.38	.40	.14
$NaH_2PO_4.H_2O$	.41	-	-
$Na_2H\ PO_4.2H_2O$	-	.045	-
$Na\ HCO_3$	1.00	.35	.20
$KH_2\ PO_4$	-	.06	-
$MgCl_2.6H_2O$	-	.10	-
$MgSO_4.7H_2O$	.23	.10	.39
Sucrose	1.00	1.00	-
H_2O (mls)	1000	1000	1000

The Frog ringer solution and Cortland Salt Solution were made up from standard reagents. In each case their pH was checked with universal indicator paper and found to be between 7.0 and 7.5. The Hank's Salt Solution was obtained in a dried form and made up according to the manufacturer's instructions.

16. Age dependent survival and fecundity

Eight uninfected B.rerio in the 28-32 mm length class were each infected with 14 adult flukes and maintained at 23°C.(Methods 7). The number of flukes present was determined on at least five occasions in each successive seven day period and counting was continued until three consecutive counts indicated that no flukes were present. The rate of egg production was assessed at least once in each successive seven day period.

17. The effect of the fluke counting method on age dependent survival

To see if the fish handling techniques or the anaesthetic, MS 222, influenced age dependent survival, six uninfected fish in the 28-32 mm length class were each infected with 14 flukes (Methods 10) and maintained at 23°C.(Methods 7). The number of flukes present was assessed (Methods 11) only once in each successive seven day period.

18. Temperature dependent survival and fecundity

Groups of eight uninfected B.rerio in the 28-32 mm length class were infected with 14 adult flukes per host (Methods 10). One group was held at each of the following temperatures:- 17°C., 19°C., 21°C., 23°C., 26°C., 29°C., 32°C., 35°C.. The number of flukes present was determined on at least five occasions during each week and the egg production assessed at least once in each successive seven day period. The results from the age dependent survival and fecundity experiment provided the 23°C. data.

19. Density dependent survival and fecundity

In all these experiments the temperature was maintained at 23°C. All the host used were in the 28-32 mm length class at the commencement of the experiment. Counting of the numbers of flukes present (Methods 11) was continued until at least two consecutive counts indicated that there were no flukes present.

Infections at parasite densities of 1 fluke per fish (39 replicates), 2 flukes per fish (11 replicates), 30 flukes per fish (8 replicates) were established. At least five times in each successive seven day period the population density was assessed (Methods 11). At least once in each of these periods the egg production of some of the fish at each initial parasite density was assessed (Methods 13) (8 fish at a density of one fluke per host, 7 at 2 and

8 at 30). The results from the age dependent survival and fecundity experiment provided the 14 fluke per host data.

Five B.rerio were placed individually in pots with 185 cercariae (Methods 10) giving an average infection of 72.4 adult flukes ± 16.47 (95% confidence limits) and the number of flukes was assessed at least five times in each successive seven day period. The egg production of the flukes on all these fish was assessed at least once in each successive five day period.

Six B.rerio were placed in pots with 370 cercariae under the conditions described previously giving average infection levels of 145.8 adult flukes per host ± 24.3 (95% confidence limits) and the number of flukes assessed at least six times in each successive seven day period. Egg production was assessed at least once in each successive five day period.

20. Survival and fecundity in reinfected B.rerio

Within three days of at least two consecutive counts indicating that no flukes were present some hosts were reinfected with T.patialense at the same densities as in their original infections. All these hosts were in the 28-32 mm size class at the time of reinfection.

Five of the eight hosts from the age dependent survival and fecundity experiment were reinfected with 14 flukes per host. The maintenance and determination of survival was the same as in the primary infection. Fecundity was not assessed.

Five of the six hosts from the group infected with an average of 145.8 adult flukes per host were placed in pots containing 370 cercaria producing an average level of infection of 132.4 flukes per host ± 13.6 (95% confidence limits). The maintenance and determination of survival and fecundity was the same as in the fishes primary

infections.

21. Survival of flukes transplanted to previously uninfected fish

Seven day old flukes were removed from B.rerio (Methods 12) which as been infected by placing them in pots containing 37 cercariae and kept at 23°C. As each fluke was removed an attempt was made to position the fluke under the scale of a previously uninfected B.rerio using the microhook round which the fluke was wrapped. Both fish were anaesthetised with the donor fish lying adjacent to the uninfected fish. If the fluke was not successfully inserted after 60 seconds it was discarded.

Six hosts were infected with an average of 7.4 flukes ± 4.2 (95% confidence limits). The number of adult flukes on each host was assessed (Methods 11) on at least five occasions in each successive seven day period. The temperature was maintained at 23°C.

22. The effect of host size on fluke survival at 23°C.

All the fish in this experiment were maintained at 23°C. (Methods 7). Four B.rerio in the 8-12 mm length class at the time of infection and had been laboratory bred (Methods 8) were infected by placing them in pots containing 37 cercariae (Methods 10). The number of flukes surviving was assessed daily (Methods 11). When at least two consecutive counts failed to find any flukes the fork lengths of the fish were again determined (Methods 9).

The survival of flukes on fish in the 16.1 - 20 mm length class (4 fish); the 20.1 - 24.0 mm length class (4 fish); the 24.1 - 28.0 mm length class (11 fish) and the 32 mm plus length class (5 fish) was also assessed. All these fish were obtained from stock tanks and were infected with 14 flukes (Methods 10). The number of flukes surviving was assessed on at least three occasions in each

successive seven day period. When at least two consecutive counts failed to detect any flukes the lengths of the fish were again assessed. The eight fish from the age dependent survival experiment formed the 28-32 mm length class.

23. The effect of cyclic illumination and temperature regimes on egg production

An automatic egg collecting device was employed in this experiment to investigate the effect of cyclic changes of illumination and temperature on egg production.

The apparatus was based on a Shandon-Elliot Histomat (fig. 5) (Shandon Elliot Ltd). This laboratory carousel is designed for automatic slide staining by moving the specimens through a series of pots containing staining and processing solutions. To do this it has a hinged arm lying above a circle of 12 containers. Governed by a timing device the arm hinges upwards, lifting the specimen from its container and rotating until the specimen is held over the next container. The specimen is then lowered into the container. The time spent in the containers and the duration of the entire cycle can be varied within wide limits.

For use in assessing egg production a rectangular cage was made from 4 mm polythene mesh with dimensions of 5x4x4 cms and was suspended from the moving arm of the Histomat. The timing system was adjusted so that the cage spent two hours in each of twelve 600ml crystallising dishes.

When a fish infected with T.patialense was placed in the cage, eggs produced by the flukes fell through the plastic mesh into a crystallising dish containing 400mls of tap water, in which it was suspended. After two hours the apparatus automatically moved the cage to the next dish until all 12 pots had been utilised. This

FIG.5.

Legend for figure 5

Modified Shandon Histomat for assessing the egg production of adult T.patialense.

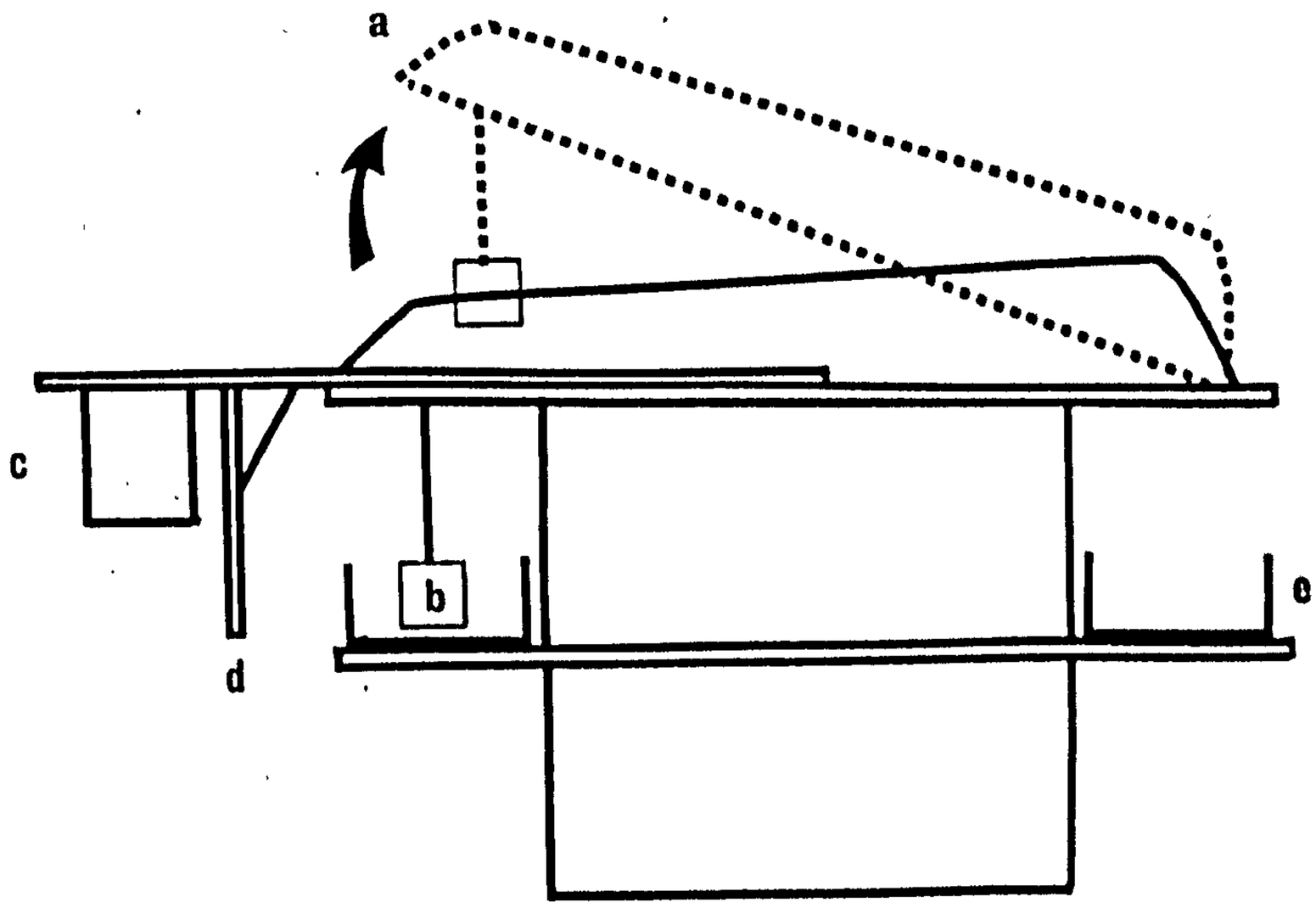
a. Moving arm of Histomat

b. Cage for infected B.rerio suspended from arm

c. Light source

d. Perspex heat shield

e. Replacement collecting dish


a
c
b
d
e

process enabled the egg output during each two hour period to be assessed (Methods 13).

The carousel was placed in a small chamber which was kept at 23°C. by a thermostatically controlled fan heater during the course of the experiments, to determine the effects of cyclic changes in illumination.

In the experiment to determine the effect of cyclic changes in temperature, a second thermostatically controlled heater was set at 26°C. and switched on for a 12 hour period in every 24 hours by means of a time clock. The commencement of this period of increased temperature was set to coincide with one of the arm movements of the Histomat.

To provide a controlled light environment an eight watt fluorescent light (Minipack eight watt fluorescent fitting, Thorn Electrical Ltd) was fitted to the circular top of the Histomat. As this circular top rotates with the arm the light remained in a constant position in relation to the experimental cage. A perspex heat shield was placed between the fluorescent tube and the experimental cage to reduce heating effects of the light to a minimum.

A control experiment was carried out to investigate this heating effect. With the arm held in a constant position the temperatures of all 12 dishes were measured after six hours of darkness, and again, after four hours illumination.

The fluorescent tube was connected to a time clock (Paragon Time Control, Gallenkamp Ltd). In the cyclic temperature experiment illumination was held constant. In the experiments to investigate the effect of cyclical illumination changes, the light was switched off for a 12 hour period in every 24 hours using the time clock. The changes in illumination were set to coincide with the change between two dishes.. As a control these experiments were repeated in condi-

tions of constant illumination.

The pots were replaced once every 24 hours by fresh ones. This change always took place during periods of illumination and at the end of the Histomat's cycle, which was at 12.00 hours. After replacing the pots with fresh ones a new 24 hour cycle was commenced.

The experimental fish was infected (Methods 10) immediately prior to the commencement of each experiment and as soon as the adult parasite population had been counted (Methods 11) it was placed in the experimental cage. The experimental fish were not fed during the experiment. At the termination the number of flukes was again assessed.

24. Growth of the adult parasite

24 a. The effect of density on growth

The growth in width of adult parasites was investigated at three densities. Twenty four B.rerio were infected with 14 flukes each, 16 with 30 flukes and four using 370 cercariae resulting in an average infection of 124 flukes (Methods 10) and maintained at 23°C. under standard conditions (Methods 7).

The flukes from six fish infected with 14 flukes,and four infected with 30 flukes, were removed (Methods 12) every seven days for four weeks. The flukes from two of the heavily infected fish were removed after seven days and from the remaining fish after 14 days.

On removal the flukes were fixed in 10%w/v formalin solution and placed on microscope slides in groups of five or six. Coverslips were placed on the slides and excess liquid drawn out from under the coverslip by gently placing a tissue against each side of the coverslip in turn. The width of the parasites at the widest point was then measured at x100 magnification using a calibrated microscope eyepiece micrometer. One hundred and fifty cercariae were obtained

from infected snails (Methods 4). Approximately two thirds of the cercariae were fixed in 10% formalin. The widths of these, and the remaining, live cercariae were measured in the manner described above. Any cercariae which were folded and not flattened were ignored, giving 90 measurements of fixed, and 23 of unfixed, cercariae.

A control experiment was performed to determine the accuracy of the measuring techniques described above.

24b. Determination of the accuracy of the measuring techniques

Two B.rerio were infected with 100 cercariae giving infections of 39 and 46 adult flukes. After seven days 36 flukes were removed from each fish. Flukes were placed six to a slide and their widths determined in the manner described above. For the flukes from each fish an analysis of variance was carried out to determine whether there was a significant difference between the widths of the flukes from different slides.

24c. The development of the vitelline glands

A diazo technique (Johri & Smyth, 1955) was utilised to localize phenolic substances present in the vitelline glands of T.patialense. The stain used, Fast Scarlet Salt GG (Solmedia Ltd), had been previously shown to react readily with such substances in T.patialense (Miss N.A.Moloney, personal communication).

The staining technique used was a modified version of that used by Johri & Smyth (1955).

a) Specimens fixed in 70% alcohol for 15 minutes

b) Transferred to water after 10 minutes

c) Transferred to a 1% solution of Fast Scarlet Salt GG in distilled water

The stain was freshly prepared and filtered before use. The

specimens were stained for exactly two minutes.

d) Washed in tap water for five minutes

e) Dehydrated, cleared and mounted. For ease of handling the specimens were contained in specially designed staining chambers for steps a-d. These were based on cylindrical E.M. casts (L.K.B. 4885-01) (see fig. 6c). The bottoms of the casts were sliced off and holes bored in the lids with a cork borer. Spare bored lids were used to fit over the other end of the casts. The functions of the lids was to hold 2 cm^2 pieces of polythene mesh (3.3 meshes /mm) over the ends of the staining chambers thus enabling liquids to pass freely in and out of the staining chambers. Attached to one of the lids were 15 cm lengths of stainless steel wire enabling manual agitation of the chambers during the staining process. Ten to twently flukes were placed in each chamber.

Fourteen B.rerio were infected with 14 adult flukes removed from each of two of the infected B.rerio 1, 2, 4, 7, 10, 15 and 20 days post infection. The timing of the infections was staggered so that all the flukes could be removed and stained on the same occasion.

The intention had been to use a Vickers M86 Scanning Microdensitometer to quantitatively determine the amount of stain in each fluke and hence estimate the phenolics in the vitelline system. It was found impossible to obtain repeatable results using this technique. Instead the stained flukes were photographed using 35 mm black and white film and A4 size prints made, taking care to keep a constant negative-to-print magnification. The images of the flukes were then cut out and the weight of the fluke-sized piece of print determined. The black stained areas of the flukes on the prints were then cut out as accurately as possible using dissecting scissors and weighed. The flukes were labelled with a code unknown to the "dissector" in an

FIG.6.

Legend for figure 6

A. Measurement of fish length. The distance between the two arrows was recorded.

B. Hook for removing adult T.patialense from under the scales of the fish host.

C. Exploded view of staining chamber for adult T.patialense.

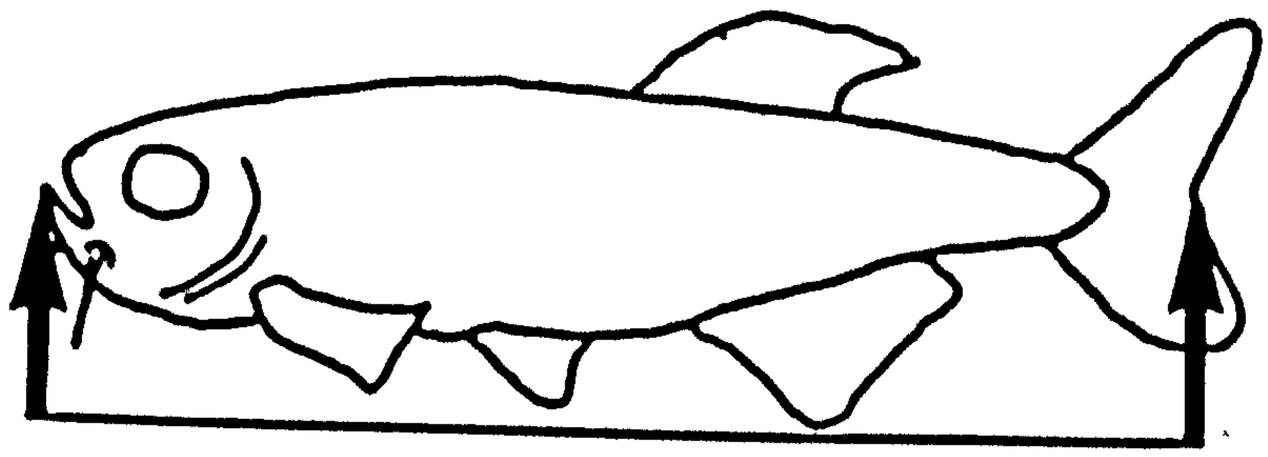
a. Stainless steel handle

b. Rings cut from the lids of E.M. casts

c. Nylon mesh

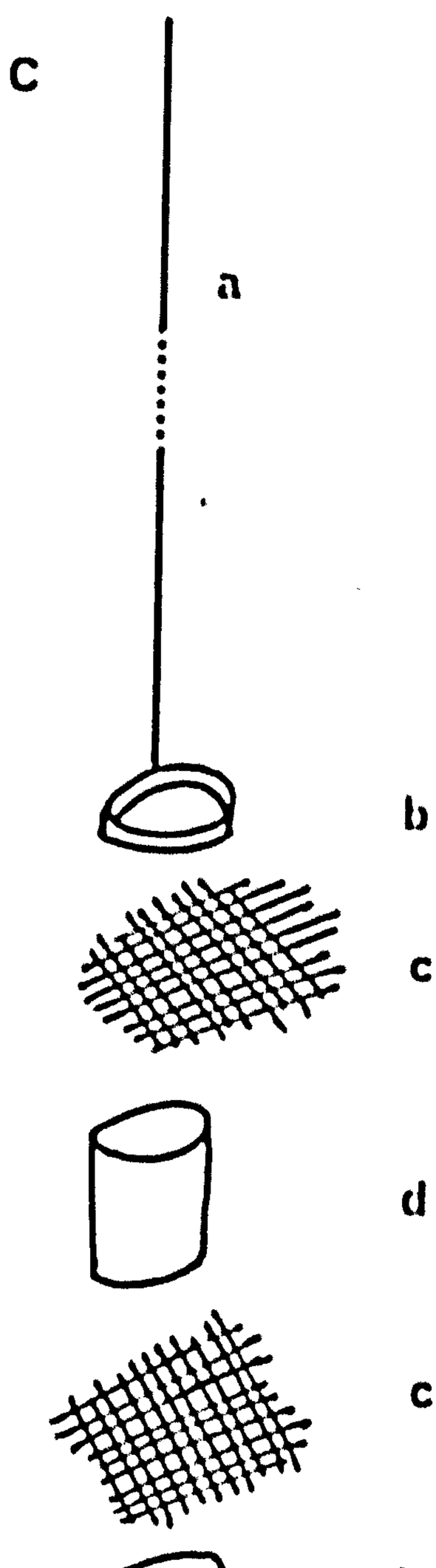
d. Body of E.M. cast

A



B

C



effort to reduce bias.

Calibration was achieved by photographing a measuring slide at the same magnification as the flukes enabling a relationship between weight of photographic paper and area to be found. The thickness and density of the photographic paper was assumed to be constant.

25. Assessment of reproductive abnormalities in adult flukes

The flukes on five of the fish in the 14 fluke per host, age dependent survival experiment (Methods 16), were observed for signs of two types of reproductive abnormality each time that the number of flukes was determined (Methods 11). These were the presence of an amorphous mass of tanned vitelline material collected in the ootype and/or the presence of one or more tanned eggs in the uterus.

Also, when tanned eggs were observed in the uterus of a fluke in the one fluke per host, density-dependent egg production and survival experiments (Methods 19), additional experiments to determine egg production using this host and parasite, were performed.

26. Survival of adult flukes in vitro; non-sterile conditions

5 ml crystallising dishes were filled with 4 mls of water or saline. The temperature of the dishes was adjusted to 23°C in a cooled incubator. Between five and thirty adult T.patialense removed from their fish-hosts (Methods 10, 12) were placed in each dish and then incubated at 23°C under conditions of constant illumination. The dishes were covered in Parafilm (Gallenkamp Ltd) to prevent evaporation. The water, or saline, was changed once during each successive 24 hour period. Periodically the basins were checked using a binocular microscope at approximately x12 magnification. Any dead flukes were removed from the dish using a Pasteur pipette.

Death was defined as a state in which three contacts with a microhook (Methods 12) failed to elicit any visible response by the fluke.

26a. Survival of adults under five minutes old

B.rerio were infected by placing them in dishes containing cercariae (Methods 10) for two minutes. The fish were immediately anaesthetised (Methods 11) and young adult T.patialense removed (Methods 12) for the next three minutes. The flukes were transferred (Methods 12) to 5 ml crystallising dishes. Five dishes contained tap water and five, full strength, Cortland Saline.

26b. Survival of three day old adult T.patialense

Three day old adult flukes were placed in 5 ml crystallising dishes. Five contained full strength Cortland Saline. Single dishes contained tap water; tap water containing 1:10,000 w/v MS222 solution; full strength Frog ringer and full strength Cortland Saline made up with tap, rather than distilled, water.

26c. Survival of eleven day old adult T.patialense

Eleven day old flukes were placed in 5ml dishes. Five contained tap water and five contained 10% and 50% strength Cortland Saline.

27. Egg production by adult flukes in vitro using non-sterile conditions

5ml crystallising dishes were each filled with 4mls of full strength Cortland Saline. Fourteen day old adult flukes were removed from infected B.rerio (Methods 12) and 20 were placed in each dish. Before the removal of the flukes the fish had been maintained in natural lighting conditions at 23°C. The flukes were removed

midway through the light period (Methods 12).

The dishes were incubated at 23°C in cooled incubators. Five dishes were incubated in continuous light and five in continuous darkness. After six hours the pots were removed from the incubators and the number of eggs was counted by direct observation using a binocular microscope at x16 magnification. The number of flukes containing tanned eggs in the uterus and the number of dead flukes was also counted.

Another six pots were incubated in constant light and the number of eggs produced was counted every hour.

28. The decaudation of cercariae and their survival in vitro in non-sterile conditions

A simplified version of the method previously described (Howells et al, 1974) for the decaudation of schistosome cercariae was employed to decaudate cercariae of T.patialense. Freshly shed cercariae were placed in 30mls of either, tap water, or, Cortland Saline, in a 50ml crystallising dish and cooled to 3-4°C. The dishes were then covered in Parafilm and shaken hard for 20-30 seconds. This procedure was found to remove 100% of the tails from the cercariae.

The decaudated cercariae were immediately placed in 5ml dishes. Five dishes contained tap water and five contained full-strength Cortland Saline.

29. Survival of adult flukes in vitro in sterile conditions

To culture adult T.patialense a batch of 10-50 adult flukes were removed from infected B.rerio (Methods 12) and placed in a 30ml sterile polystyrene vial. The vial contained approximately 15mls of Hank's solution (Difco Ltd) containing Phenol Red indicator and

adjusted to neutral pH using CO_2 if necessary. The Hanks solution contained 100 units/ml of penicillin-streptomycin mixture (penicillin-streptomycin, 5000 I.U. 1ml. Flow Laboratories Ltd) and was sterilised by passing it through a Millex disposable filter unit (Millex 0.22 um Disposable Filter Unit, Gallenkamp Ltd) using a sterile 20ml syringe. All sterile manipulations were performed in a laminar flow hood (Slee Medical Ltd).

The vial containing the adult flukes was rotated for ten minutes on a blood cell suspension mixer (Gallenkamp). About 95% of the Hank's solution was then drawn off using a sterile pipette and replaced with fresh solution. The vial was then rotated again. The bulk of the Hank's solution was then again withdrawn aseptically and the remainder, containing the adult flukes, was transferred to a sterile Leighton tube (Gallenkamp Ltd) containing 5mls of sterile Hank's solution.

Three Leighton tubes each containing between 18 and 27 three day old adult flukes and four tubes containing between 21 and 26 eleven day old flukes were set up in the manner described above. The tubes were incubated at 23°C under conditions of constant illumination. Each tube was examined at least three times in each successive 24 hour period using a binocular microscope at x20 magnification and the number of flukes showing any movement was determined.

30. <u>Survival of cercariae in water and saline</u>

The methods used to determine cercarial survival were similar to those described by Anderson et al (1977). For each experiment, between five and ten 35ml evaporating basins were each filled with 20mls of tap water or Cortland saline (Methods 15). The temperature of the basins was then adjusted to 23°C in a cooled incubator.

Approximately ten cercariae released from M.tuberculata in the previous 15 minutes were added to each basin. The basins were covered with a sheet of perspex to reduce evaporation and incubated at 23°C in a cooled incubator in conditions of constant illumination. Periodically the basins were checked using a binocular microscope at approximately x12 magnification. Any dead cercariae were removed from the dish using a Pasteur pipette. Death was defined as being a state in which three contacts with a microhook (Methods 12) failed to elicit any movement in the cercariae. Any cercariae which shed their tails were considered to be dead as they had effectively left the cercarial population and become adults.

31. Infection of B.rerio in tap water and Cortland Salt Solution

Fourteen uninfected B.rerio in the 28-32 mm size class were infected by placing them in dishes containing ten cercariae in the conditions described previously (Methods 10). Another 14 B.rerio were infected in the same way except that the tap water was replaced by Cortland Salt Solution diluted to 50% of the strength described in Methods, 15, with distilled water.

After two hours all the fish were placed in fresh tap water and the number of adult flukes on each fish was determined (Methods 11).

CHAPTER 3

Age Dependent Survival and Fecundity

This, and succeeding chapters (4,5,6,7,10), describe some aspects of the survival characteristics of adult T.patialense. Simple deterministic and stochastic time-dependent models are extensively utilised in the presentation of these results.

Such an approach helps in the analysis of the survival data and also provides a unified conceptual framework for considering the survival characteristics of T.patialense under different experimental regimes.

In the parasitological literature, when fecundity is being investigated, total egg production of all the parasites per host is often considered. This measure of egg production has two components, egg production per parasite, and, the number of parasites per organism. Here, and in the following chapters, egg production is generally considered in terms of a rate per individual parasite, unless otherwise stated.

a) Survival

The adult fluke has a life span of about ten weeks on the surface of B.rerio in the 28 to 32 mm size class at 23°C. The observed survival characteristics of parasite populations on fish hosts with an initial parasite density of 14 flukes per host are illustrated in fig. 7. The points on the graph represent the mean number of parasites surviving at weekly intervals estimated from eight different hosts. From fig. 7 it appears that survival is likely to be age dependent. Age dependency in the survival of a organism occurs when the death rate per unit of time per organism is some function of the age of the organism.

The models used to analyse the survival data have been described by Anderson and Whitfield (1975) and Anderson, Whitfield and Mills (1977). Assuming that the death rate, μ, is approximately constant within a small time interval, where small means some minute fraction of maximum survival, the age-dependent rate of instantaneous mortality ($\mu(t)$) an be estimated using the expression

$$\mu(t) = \ln N_t - \ln N_{t+1} \qquad (1)$$

derived from a deterministic model of an experimental death process (Anderson and Whitfield, 1975). From fig. 8 it can be seen that this death rate is age, and hence, time dependent. The instantaneous death rate per adult parasite per week, versus age, illustrates the functional form of this process (fig. 8). The relationship between death rate and time can be described empirically by the following exponential model,

$$\mu(t) = a \exp(bt). \qquad (2)$$

where μ = death rate

t = time

a, b are constants estimated using a least squares regression technique (Bailey, 1959).

FIG.7.

Fig. 7

The mean proportion of flukes surviving at a series of consecutive points in time at 23°C, and with an initial density of 14 flukes per host.

1. The solid circles represent the observed proportions surviving
2. The vertical bars show the 95% confidence limits.

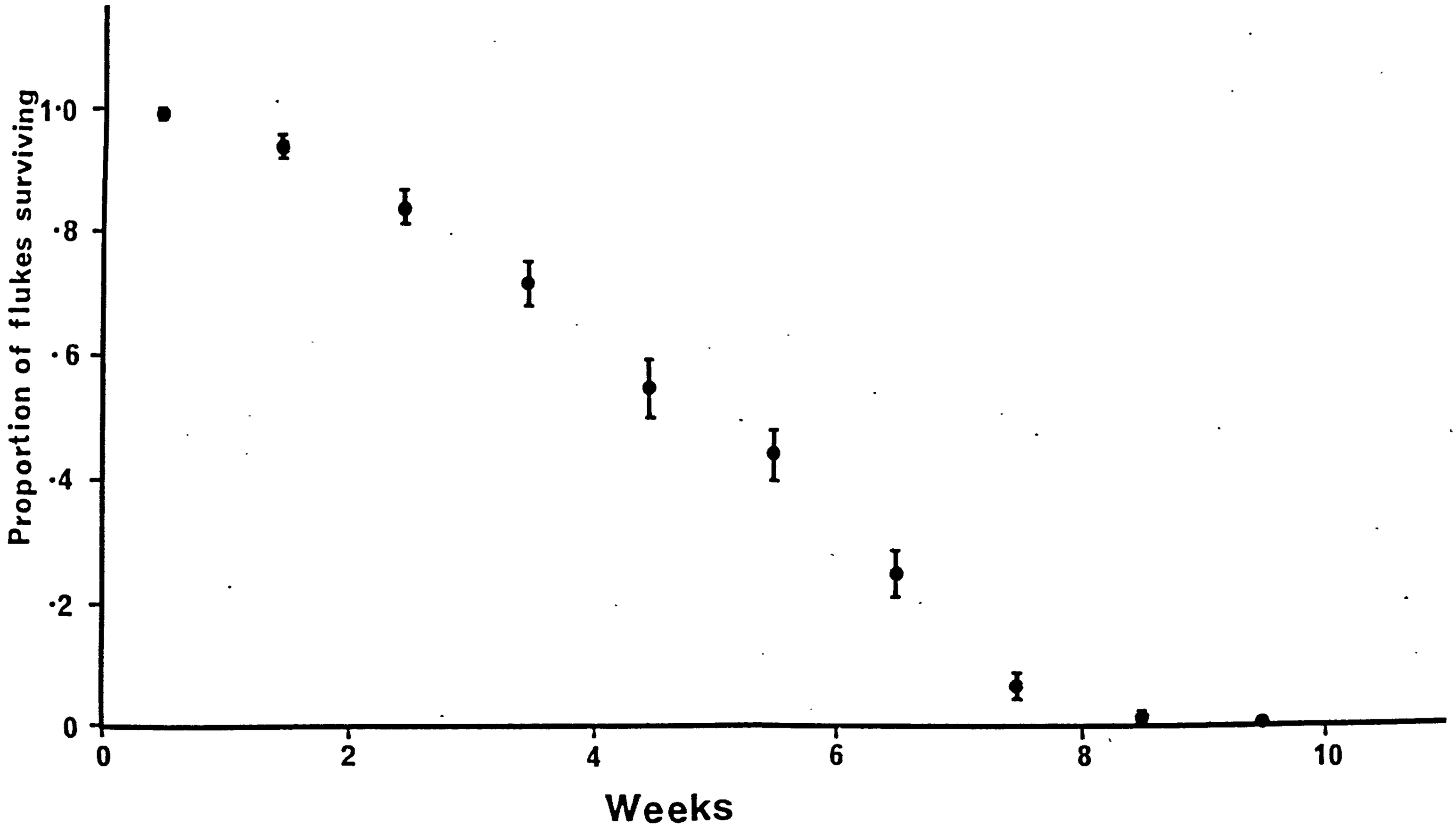

Proportion of flukes surviving
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks

FIG.8.

Fig. 8

The instantaneous death rate per week of flukes against time at 23°C and with an initial density of 14 flukes per host.

1. The solid circles represent the observed age dependent death rate
2. The solid line shows the fit of the empirical model to the observed points.

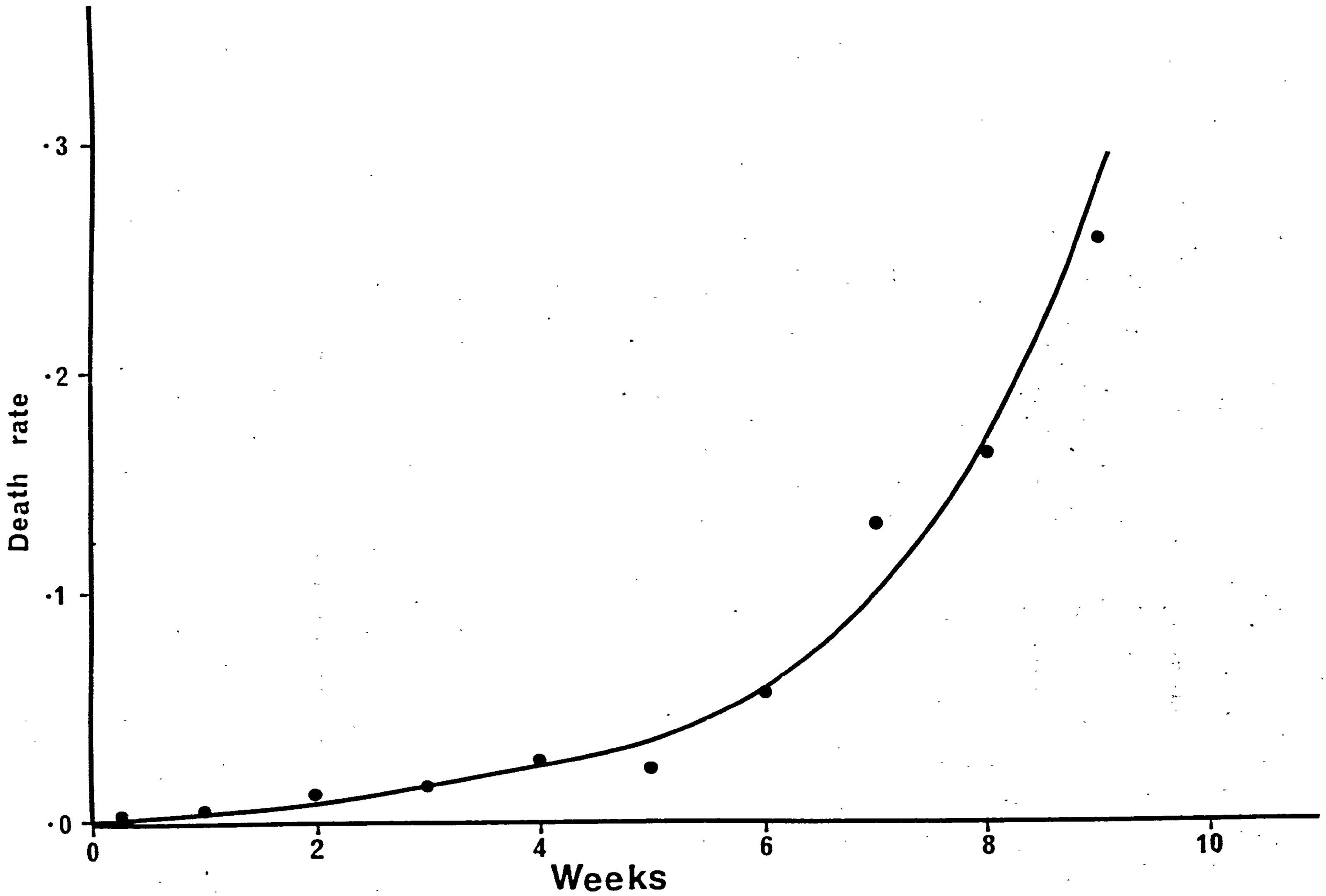
$\mu(t) = a \exp(bt)$

$a = .02803$

$b = .51291$

$r = .9822$

$P < .01$


Death rate
·3
·2
·1
·0
0
2
4
6
8
10
Weeks

The solution to this time-dependent process is given in Anderson and Whitfield (1975),

$$N_t = N_o \exp\left[- \int_o^t (v)\, dv \right] \quad (3)$$

which leads to

$$N_t = N_o \exp\left[\frac{a}{b} - \frac{a}{b} \exp (bt) \right] \quad (4)$$

The natural logarithms of the instantaneous death rates were designated as the dependent variable and the age of the organism, the independent variable, in the above linear regression analysis.

The analogous stochastic model of this, a similar process to that of the cercariae of T.patialense, has been described by Anderson and Whitfield (1975),

$$P_n(t) = \left(\frac{N_o}{N}\right) \exp\left[-N\int_o^t \mu(v)dv \right]\left[1 - \exp\left[- \int_o^t \mu(v)dv \right] \right]^{N_o - N} \quad (5)$$

Such a model takes into account the chance events involved in time of death of each adult fluke. It predicts a positive binomial distribution for the probability P_n (t) of observing N adults at time t.

The mean and variance of this distribution are given in Anderson and Whitfield (1975) and are

$$\text{Mean } (N_t) = N_o \exp\left[-\int_o^t \mu(v)\, dv \right] \quad (6)$$

which leads to

$$\text{Mean } (N_t) = N_o \exp\left[\frac{a}{b} - \frac{a}{b} \exp (bt) \right] \quad (7)$$

and

$$\text{Variance } (N_t) = N_o \exp\left[- \int_o^t \mu(v)dv \right]\left[1 - \exp\left[- \int_o^t \mu(v)dv \right] \right] \quad (8)$$

which leads to

$$\text{Var} = N_o \left[\exp \left(\frac{a}{b} - \frac{a}{b} \exp (bt) \right] \left[1 - \left[\exp \frac{a}{b} - \frac{a}{b} \exp(bt) \right] \right] \right] \quad (9)$$

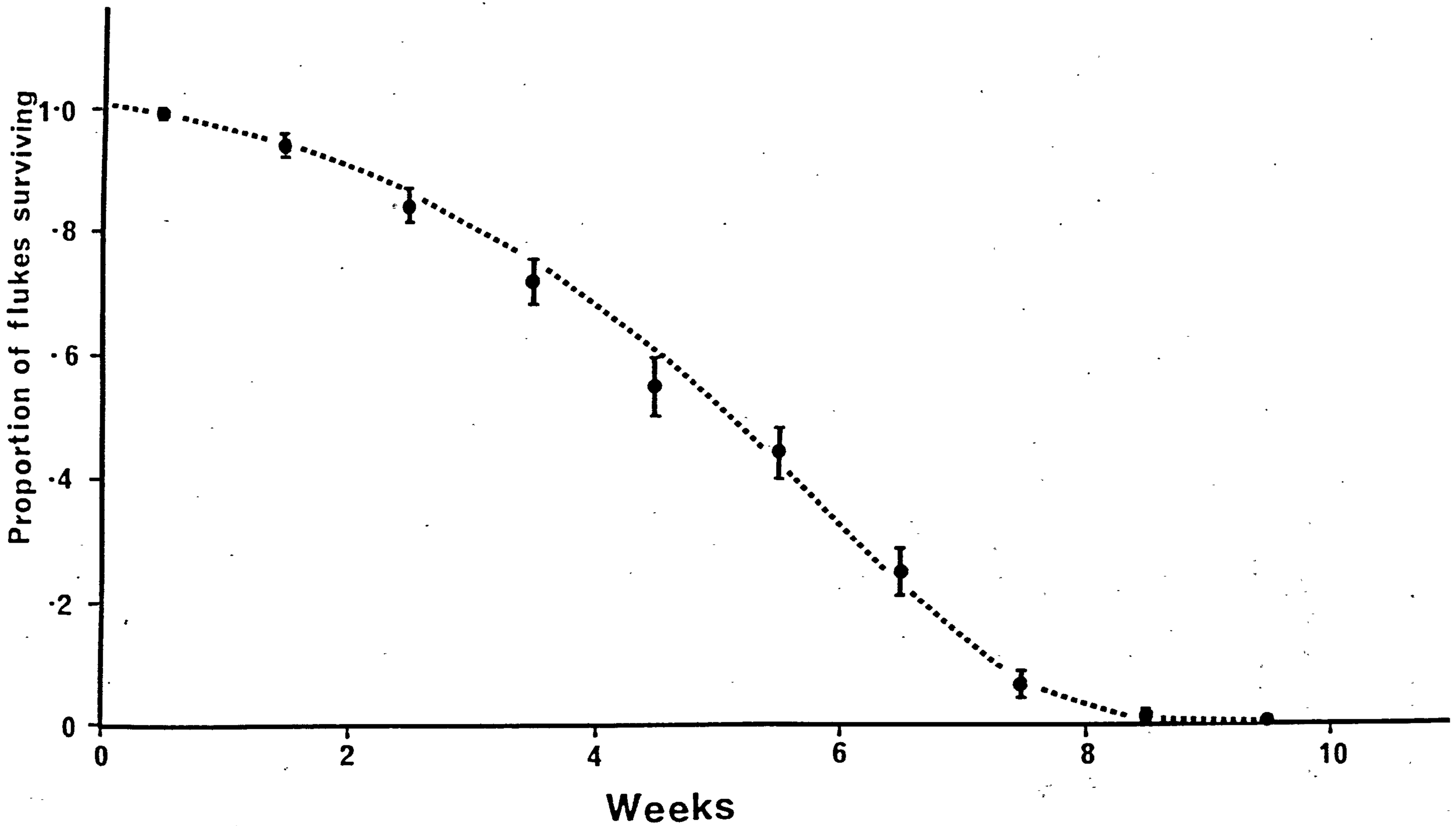
The observed means of the number of parasites surviving at various points in time show good agreement with the predictions of the stochastic model (fig. 9). The observed variances, however, are larger,on average, than those predicted by the model (equation 9) (fig 16D) for survival at 23°C. The same is true, on average, for the variances over a wide temperature range (fig. 16A - H). Obviously

FIG.9.

Fig. 9

The mean proportion of flukes surviving at a series of consecutive points in time at 23°C and with an initial density of 14 parasites per host.

1. The dashed line is the survival curve predicted by the survival model (equation 4)
2. The solid circles represent the observed proportions surviving
3. The vertical bars show the 95% confidence limits


Proportion of flukes surviving
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks

there must be an additional source of variability not accounted for in the model.

One possibility is that the conditions within the micro-environment under the hosts scales may vary from fish to fish. Due to this heterogeneity the death rate itself (μ) would be different on each fish. Thus, if it were possible to incorporate μ as a random variable into the survival model, it might give a more realistic fit to the data.

Anderson and Michel (In Press) cite variability in the size of the initial innoculum of parasites administered as a cause of extra variability in similar models. The high degree of accuracy possible in counting T.patialense on its host makes this unlikely to be so here.

b) Fecundity

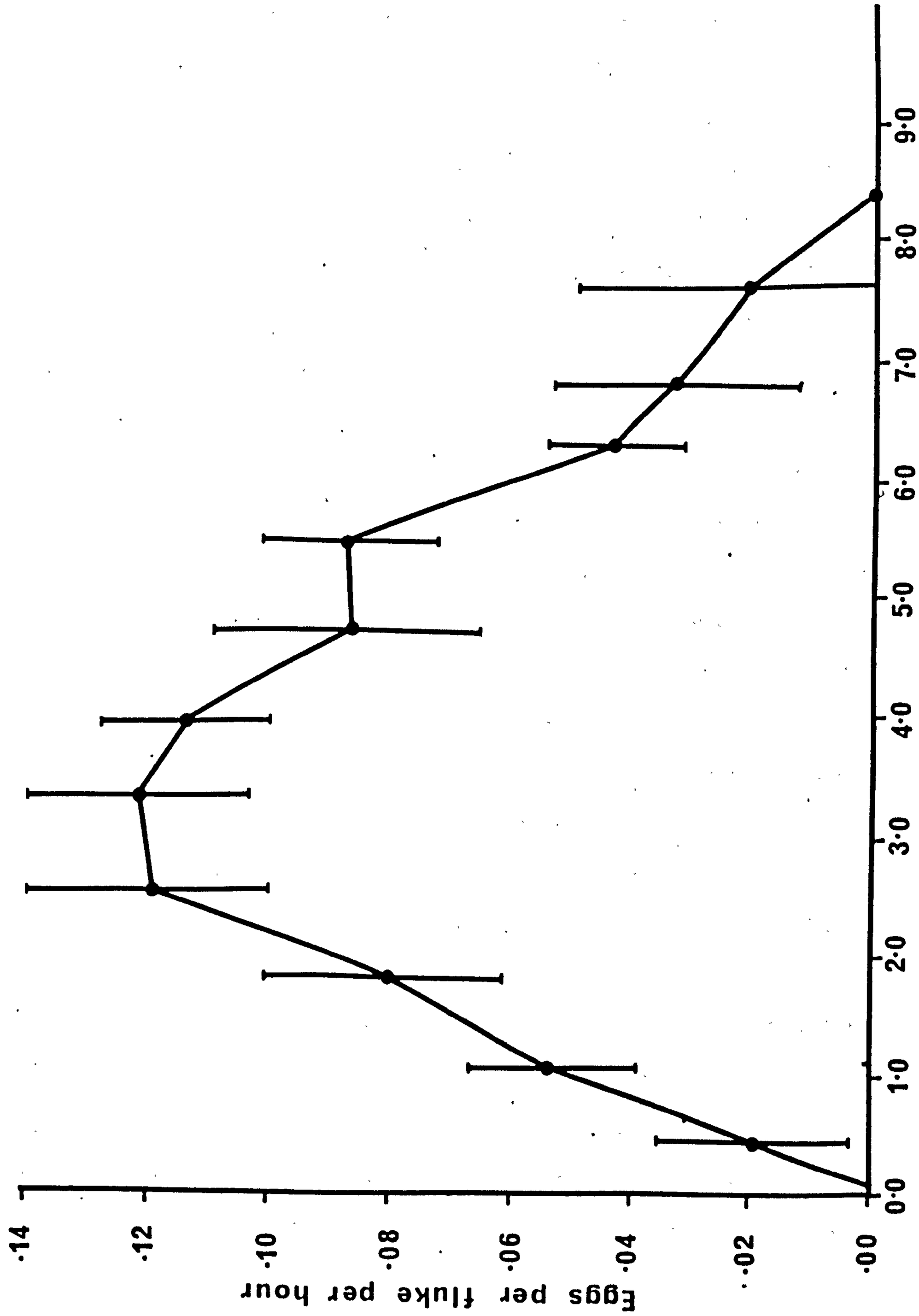
Egg production commences between days two and four (48-96 hours) post infection on B.rerio in the 28-32 mm length class at 23°C when each host carries an initial density of 14 flukes. The rate of egg production per fluke per hour on each host was determined by dividing the number of eggs produced by the average of the number of flukes on each host at the commencement and the termination of the collection period. No apparent consistent differences were observed in the rate of egg production per fluke between the parasite populations on different hosts in the experiment.

The rate of egg production per surviving fluke rises steeply for about two and a half weeks post infection. This is followed by a relatively gentle decline in the rate to about 8-5 weeks post infection when egg output ceased (fig. 10).

FIG. 10.

Fig. 10

Egg production per surviving fluke per hour against time at 23°C on fish with an initial density of 14 flukes per fish. The vertical bars show the 95% confidence limits.


Eggs per fluke per hour
.14
.12
.10
.08
.06
.04
.02
.00
Weeks
0·0
1·0
2·0
3·0
4·0
5·0
6·0
7·0
8·0
9·0

c) The effect of the fluke counting method on survival

Six hosts infected with 14 flukes each were examined only once, instead of at least five times, in each successive seven day period. The purpose of this control experiment was to discover whether the fish handling techniques, or exposure to the anaesthetic MS222, had any effect on age dependent survival.

From fig. 11 it appears that the proportions of flukes surviving at successive points in time in this control experiment, are similar to those from the original age dependent survival experiment. From the survival data for the control experiment (table 1A) the instantaneous death rates for the flukes were determined at a series of consecutive points in time (table 1B). Natural logarithmic transformations of this data were then compared with those for the original age dependent data (table 28) to see if the linear regressions for the sets of data were significantly different using the method described in chapter 6. The transformed data points and the regression lines for the original and control experiments are shown in fig. 12.

There was found to be no significant difference between either the slopes ($P > .05$) or intercepts ($P > .10$) of the regressions. Thus, if the fish handling techniques, or the use of MS222, have deleterious effects on the survival of T.patialense, those effects are not quantitatively different for frequencies of fluke counting between one and five times a week.

Using the coefficients a(intercept) and b(slope) from the linear regression fitted to the data from the control experiment, the instantaneous death rate predicted by the exponential model (equation 2) was determined at a series of consecutive points in time. It can be seen from table 1B that these predicted values are in good agreement with the observed data.

FIG.11.

Fig. 11

The proportion of flukes surviving at a series of consecutive points in time. The proportion was assessed either at least five times (open circles, dashed line), or once (solid circles, solid line) in each successive seven day period.

The vertical bars denote the 95% confidence limits for the control experiment (solid circles, solid lines). For confidence limits for the hosts assessed five times a week see fig. 7.

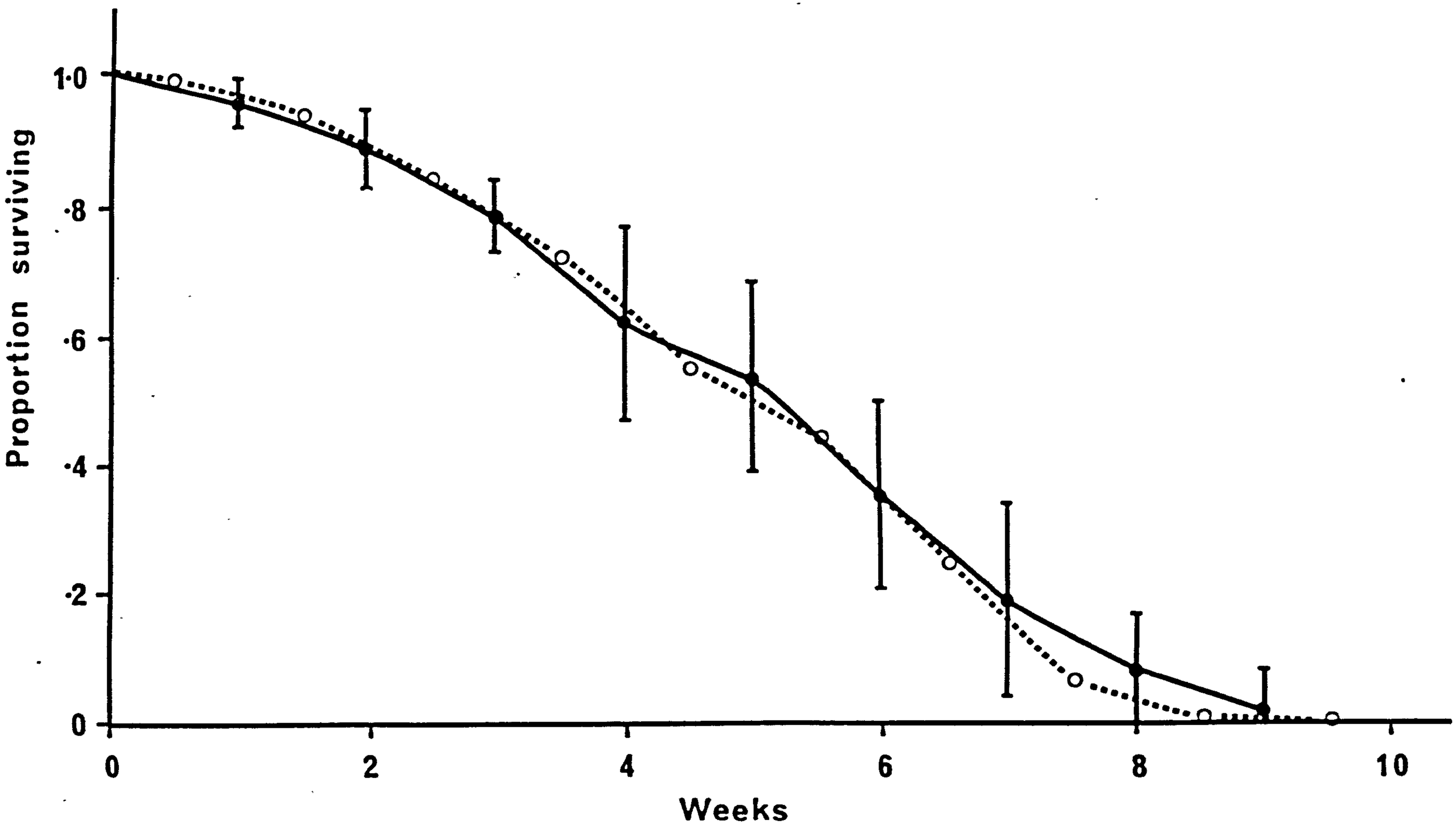

Proportion surviving
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks

FIG.12.

Fig. 12

Natural logarithmic transformations of the instantaneous death rates of flukes at a series of consecutive points in time. The open circles show the observed results for flukes where survival data was assessed at least five times a week and the dashed line a regression fitted to this data with the coefficients

a (intercept) = -3.5744

b (slope) = .5108

The solid circles show the observed results for flukes where survival data was assessed once a week and the solid line a regression fitted to this data with the coefficients

a (intercept) = -3.2189

b (slope) = .4066

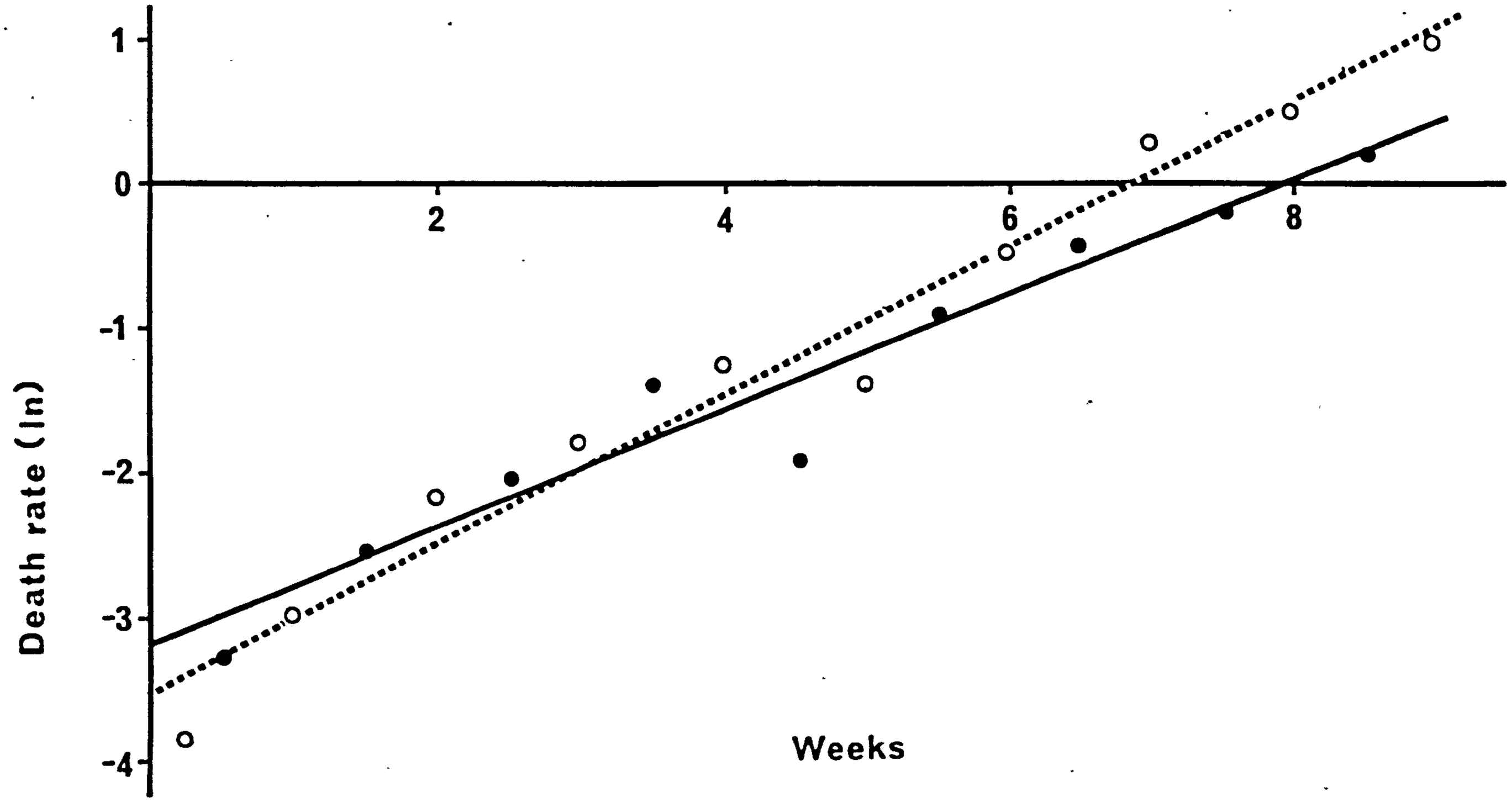

1
0
-1
-2
-3
-4
2
4
6
8
Death rate (ln)
Weeks

Table 1 A. Proportion of parasites surviving at a series of consecutive points in time.

B. Instantaneous death rates at a series of consecutive points in time.

A				B			
Time (weeks)	Proportion surviving	Standard deviation	95% confidence limits	Time (weeks)	Instantaneous death rate	Ln. of instantaneous death rate	Instantaneous death rate predicted by exponential model
1	.9643	.0391	.0411	0	.0377	-3.278	.0490
2	.8929	.0598	.0628	1.5	.0761	-2.5652	.0736
3	.7857	.0538	.0565	2.5	.1279	-2.0565	.1105
4	.6190	.1429	.1500	3.5	.2385	-1.4334	.1660
5	.5357	.1336	.1402	4.5	.1445	-1.9345	.2493
6	.3571	.1498	.1572	5.5	.4056	- .9024	.3743
7	.1905	.1475	.1548	6.5	.628	- .4652	.5622
8	.0833	.0835	.0876	7.5	.827	- .1900	.8440
9	.0238	.0583	.0613	8.5	1.2528	.2254	1.2677
					slope	.4066	.4066
					intercept	-3.2189	.0400

Table 2 Analysis of variance to compare the slopes and intercepts of ln. transformations of the instantaneous death rates of the 14 fluke per host at 23°C experiment (table 28) and the MS222 control experiment (table 1B).

	df	Σx^2	Σxy	Σy^2	Regression coefficient	Deviations from regression		
						df	SS	MS
Within normal	9	80.306	41.02	21.66	.5108	8	.70727	.0 8841
control	8	60.00	24.394	10.83	.4066	7	.9122	.1303
						15	1.61947	.10796
Pooled (W)	17	140.306	65.414	32.49	.4662	16	1.9925	.1245
				Difference between slopes		1	.3730	.3730
Between (B)	1	.006	.06	.11				
W + B	18	140.3125	65.42	32.60		17	2.098	.1234
				Between adjusted means		1	.1055	.1055

Comparison of slopes F = .3730/ .10796 = 3.4552 df.1, 15 P>.05

Comparison of intercepts F = .1055/ .1245 = .8474 df.1, 16 P>.10

CHAPTER 4

Temperature Dependent Survival and Fecundity

In attempting to gain insights into the population dynamics of T.patialense, the influence of temperature on the adult parasite on its poikilothermic host, must be investigated in a quantitative manner. Temperature can affect the ultimate egg production, and, hence, the transmission rate to the intermediate host, in two ways. Firstly, it may affect the life-span of the parasite and hence, the period available for egg production, and secondly, it may influence the actual rate of egg production. Attempts have been made to quantify the separate and combined effects of these two temperature dependent processes on adult T.patialense.

a) Survival

The series of experiments using hosts in the 28-32 mm size class, with an initial parasite density of 14 flukes per host, show that survival is characterised by temperature dependency. Temperature dependence is defined here as being a relationship where the mortality rate per individual parasite per unit time is some function of temperature (fig. 13, table 5).

23°C is the optimum temperature for survival; mortality increasing progressively at both higher, and lower, temperatures. This survival data is shown in comparative form in fig.14.

The mean instantaneous death rates at each temperature were determined as described in chapter 3, and the empirical model described in chapter 3 was used to describe the relationship between death rate and parasite age. The coefficients from the model are listed in table 3. The fit of the model to the observed data is extremely good except at 17°C, the lowest temperature, where $P>.02$ and at 35°C, the highest temperature where there were insufficient data points to calculate correlation coefficient. From the data it is clear that

FIG.13.

Fig. 13

The mean proportion of flukes surviving at a series of consecutive points in time after infection at an initial density of 14 flukes per host.

1. The solid lines are the survival curves predicted by the survival model (equation 4)
2. The solid circles represent the observed proportions surviving
3. Where present the horizontal lines denote the extent of the 95% confidence limits to the observed proportions surviving

A. 17°C

B. 19°C

C. 21°C

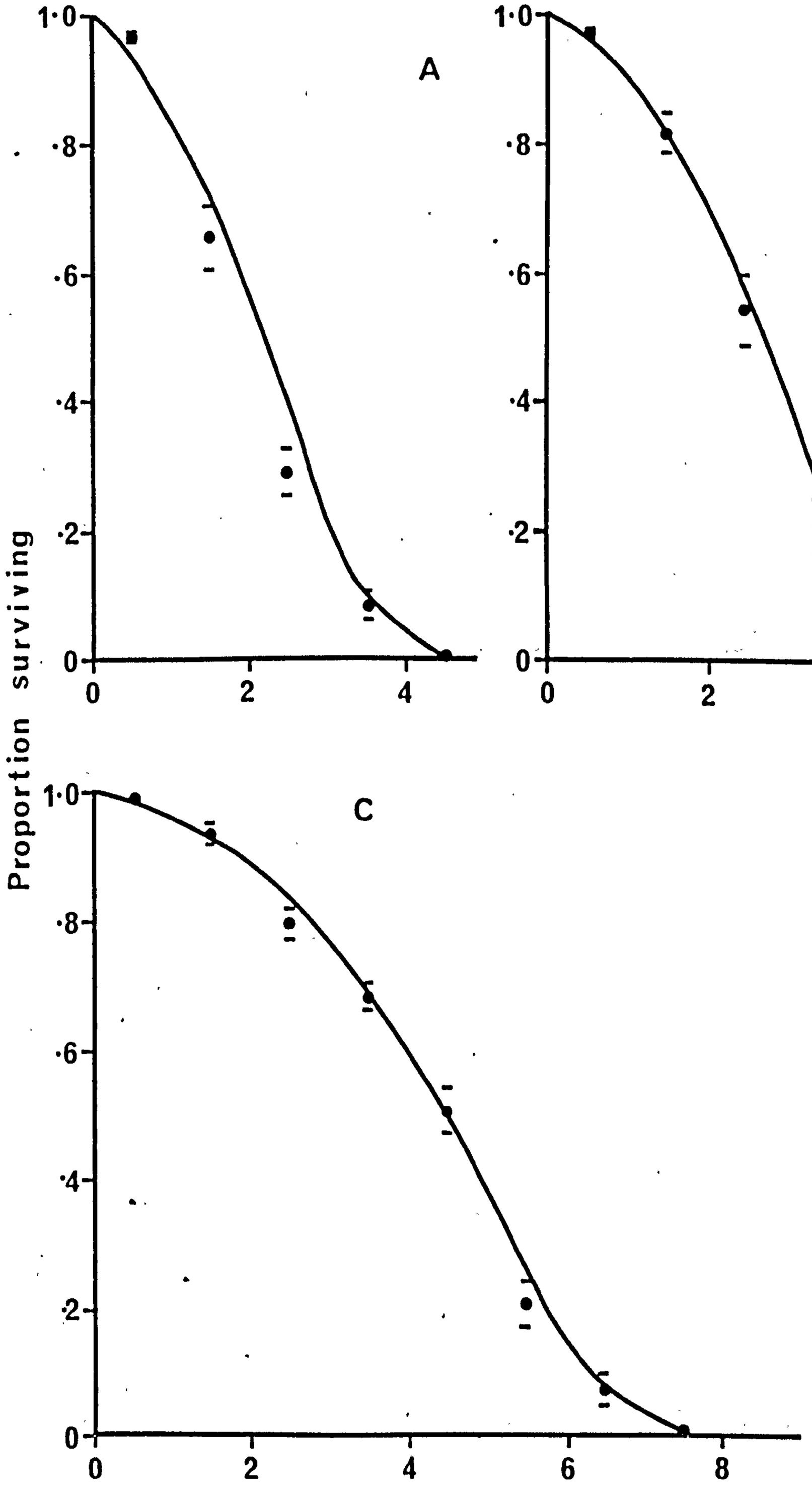
D. 23°C

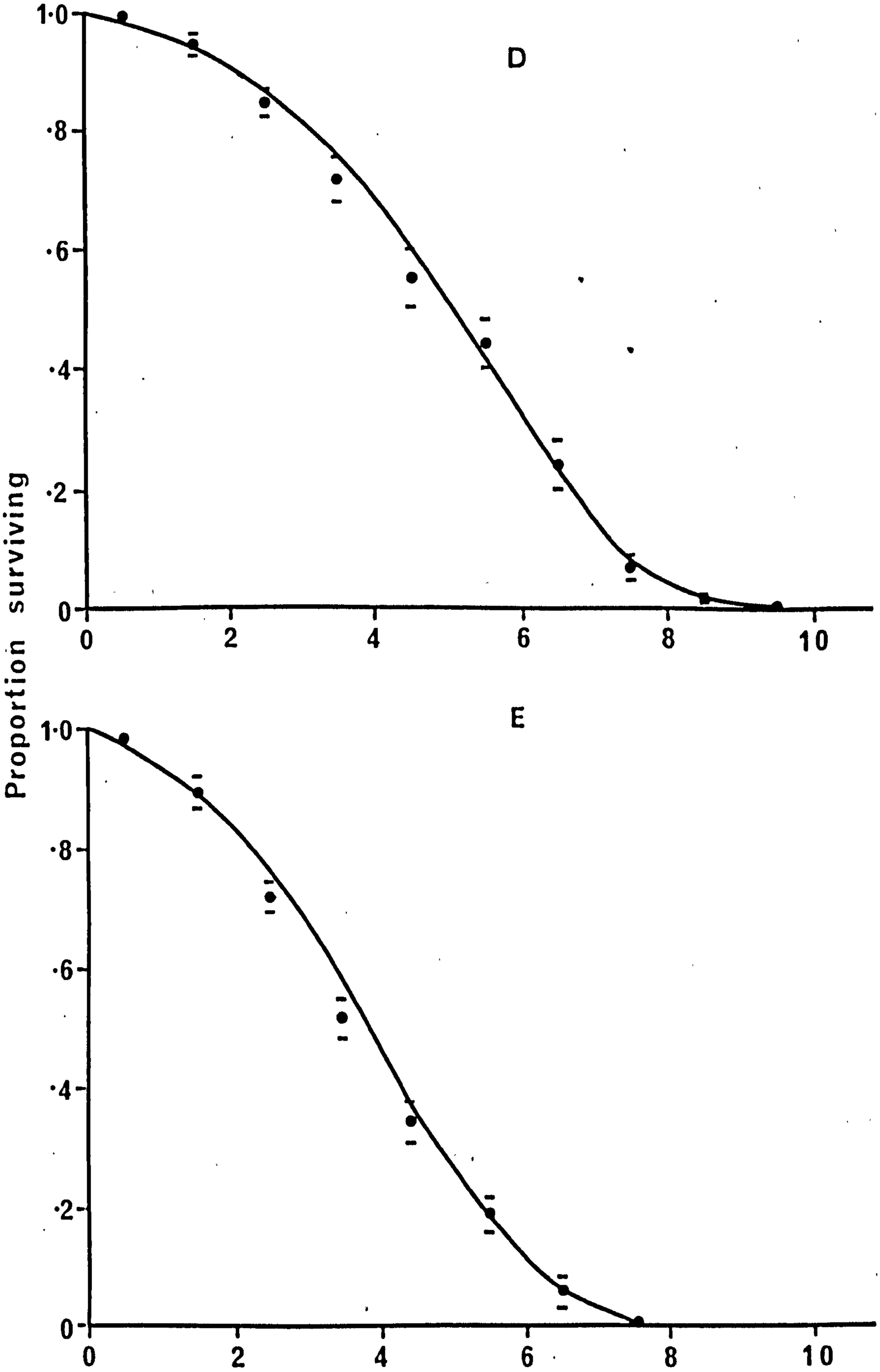
E. 26°C

F. 29°C

G. 32°C

H. 35°C


A
B
C
Proportion surviving
1·0
·8
·6
·4
·2
0
0
2
4
6
8
Weeks


D
E
Proportion surviving
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks

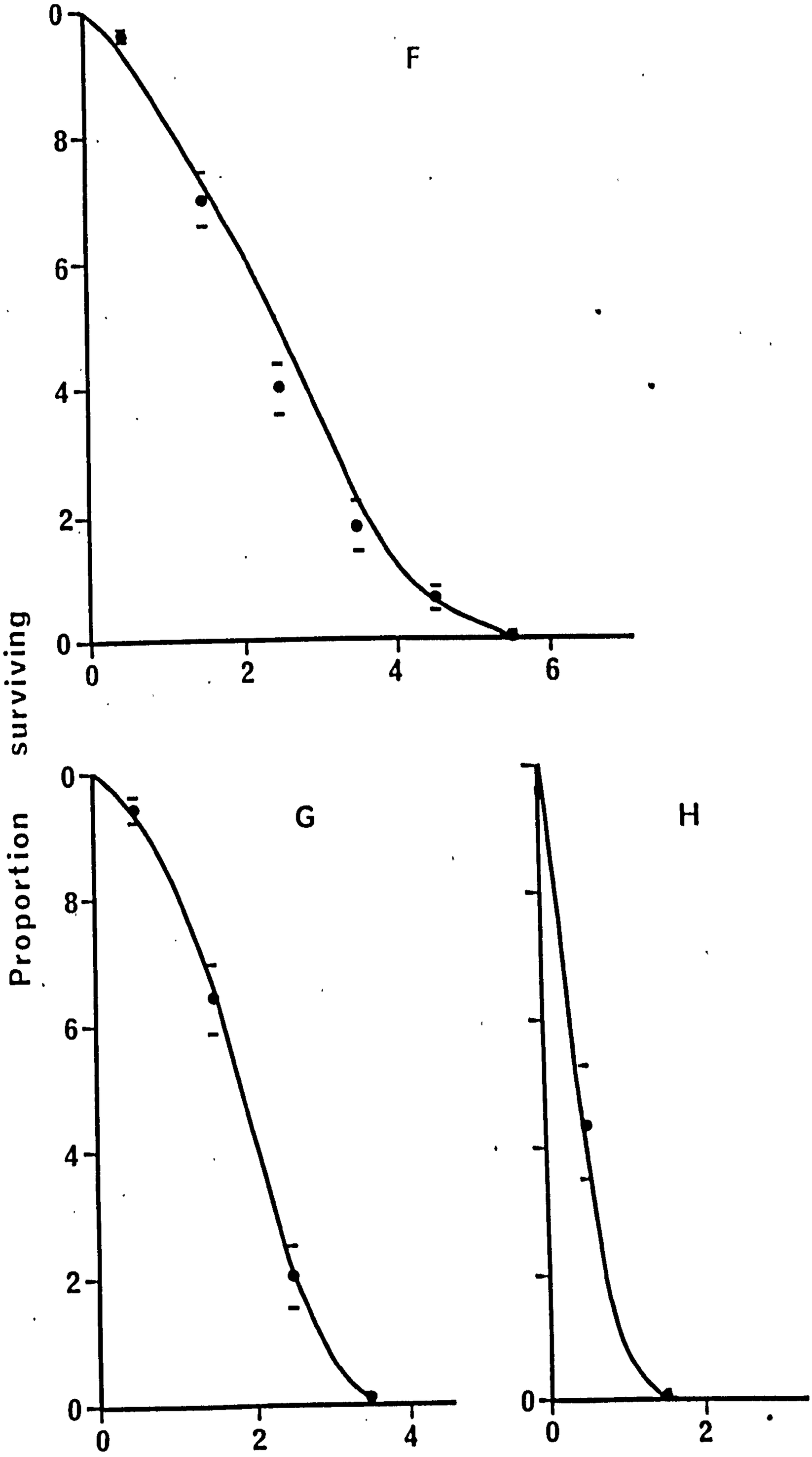

F
G
H
Proportion surviving
0
8
6
4
2
0
0
2
4
6
Weeks

FIG.14.

Fig. 14

The proportion of parasites surviving at the midpoints of successive weeks post infection shown for eight different temperatures.

1. The heavy dashes link each time point between the different temperatures.

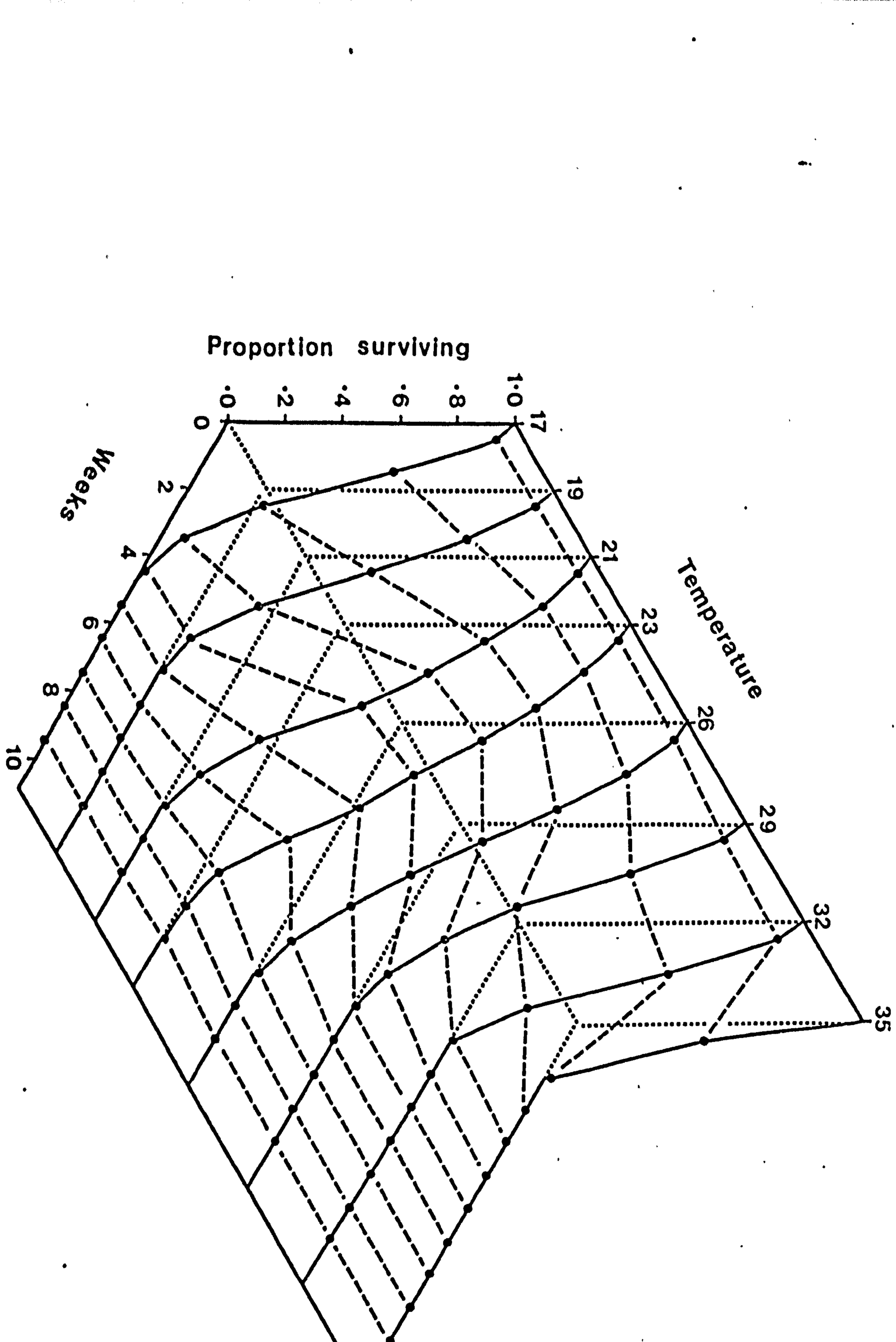

Proportion surviving
0
2
4
6
8
0
17
19
21
23
26
29
32
35
Weeks
0
2
4
6
8
10
Temperature

FIG.15.

Fig. 15

The instantaneous death rates of flukes against time at initial densities of 14 flukes per host.

1. The solid circles represent the observed instantaneous death rate.
2. The solid line shows the fit of the empirical model (equation 2) to the observed points.

A. 17°C

B. 19°C

C. 21°C

D. 23°C

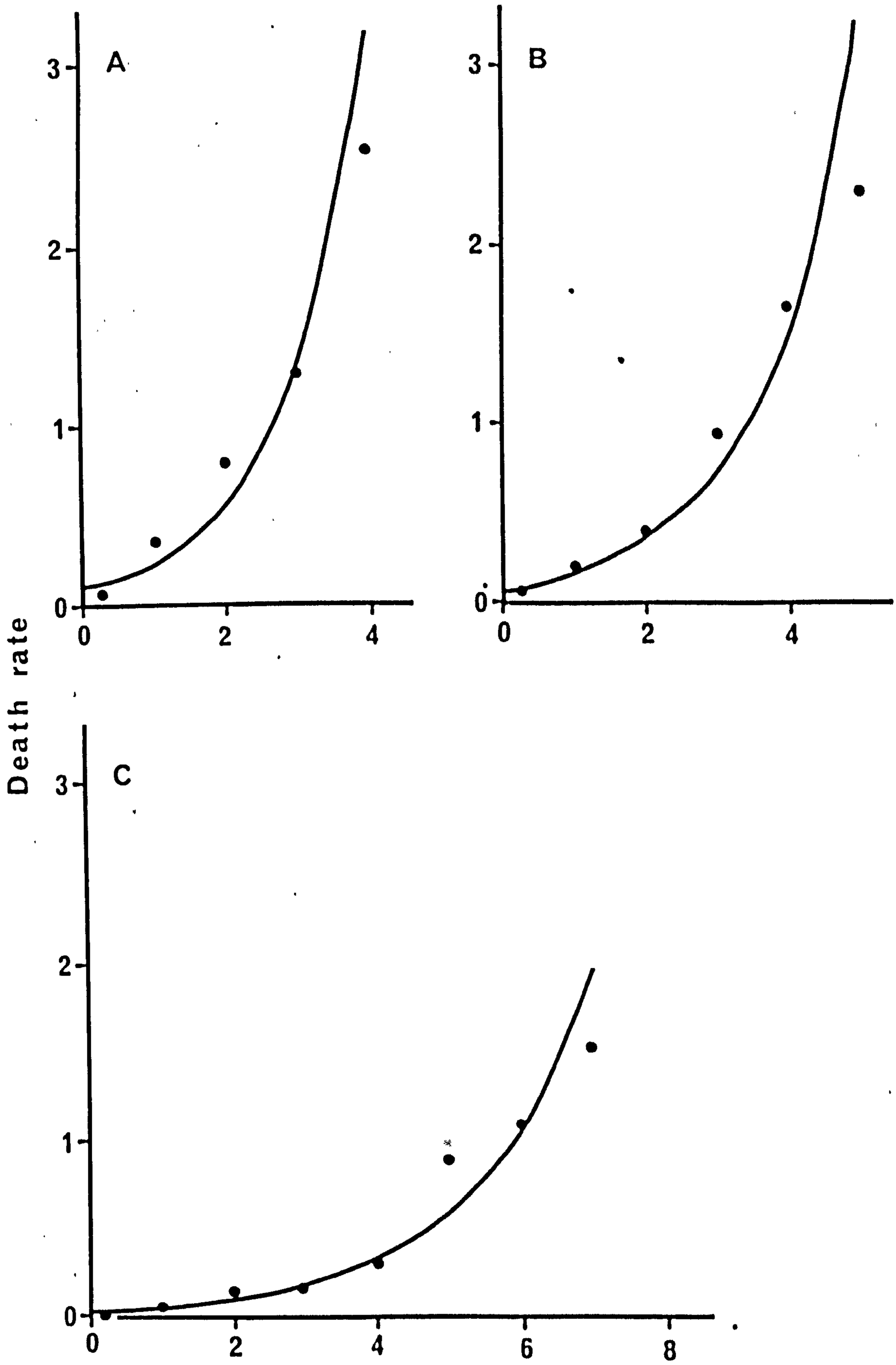
E. 26°C

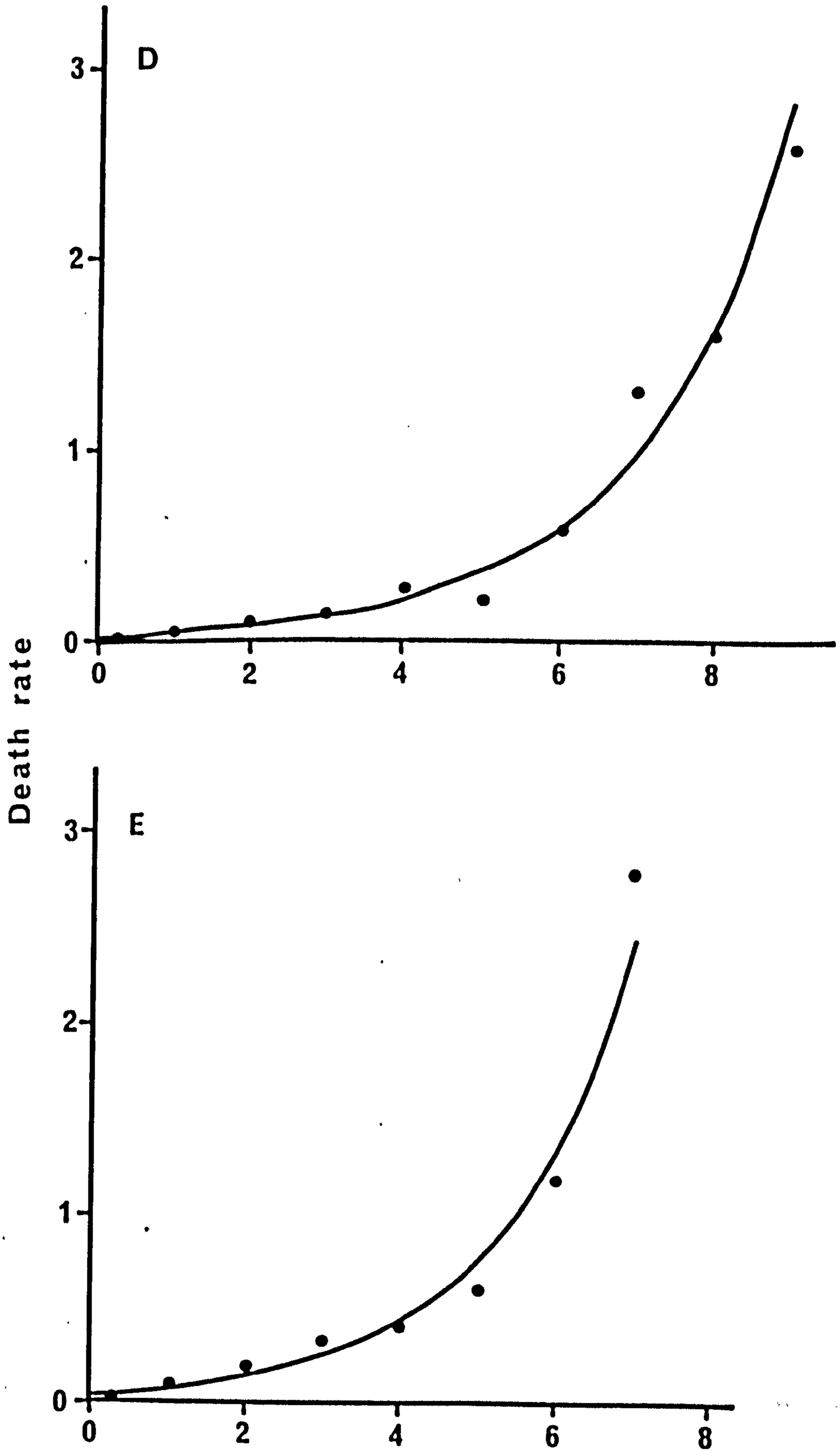
F. 29°C

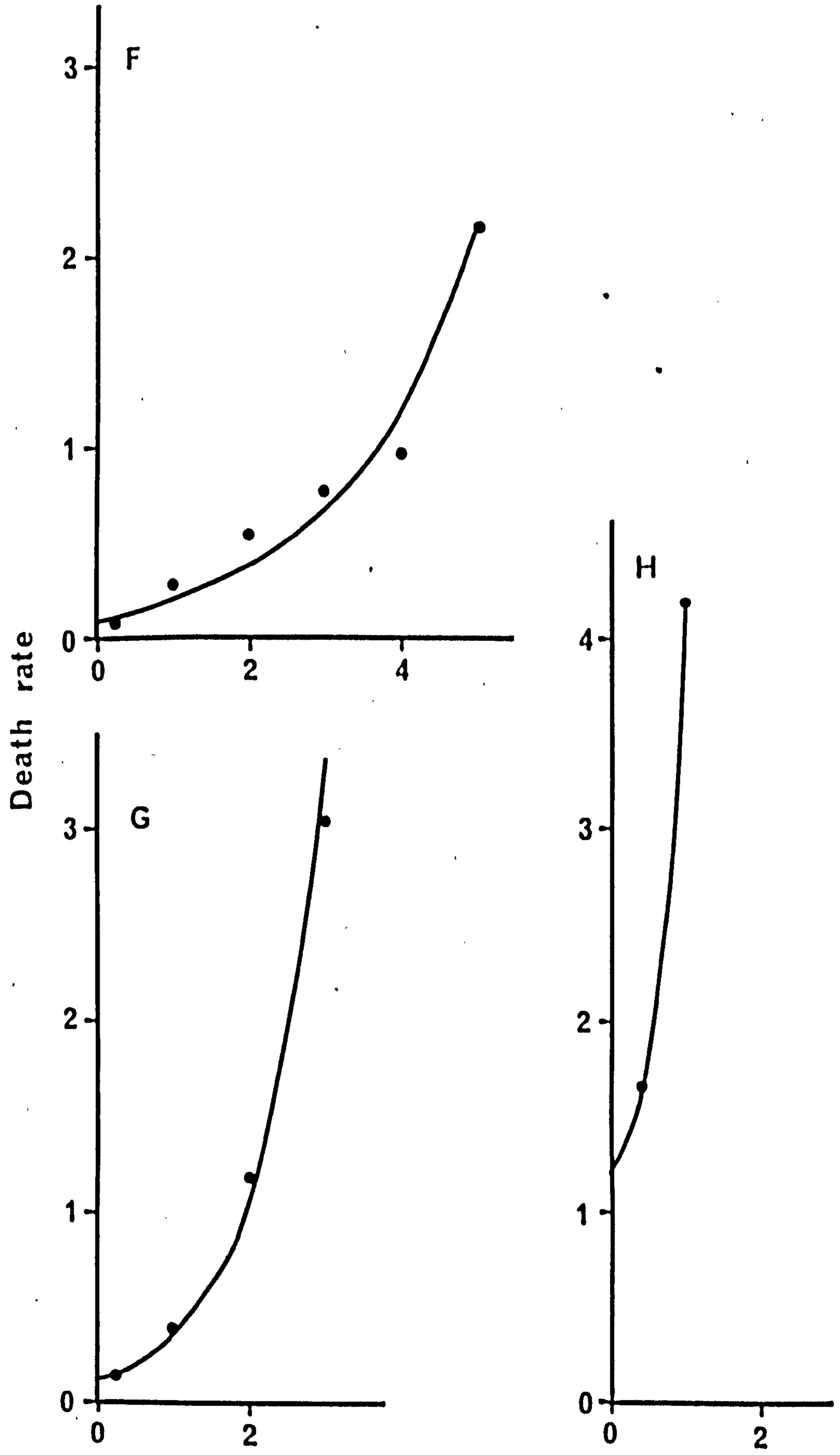
G. 32°C

H. 35°C

For the values of the constants for the model and the goodness-of-fit of the model (equation 2) to the observed points, see table 3.


A
B
C
3
2
1
0
0
2
4
6
8
Death rate
Weeks


D
3
2
1
0
0
2
4
6
8
Death rate
E
3
2
1
0
0
2
4
6
8
Weeks


F
3
2
1
0
0
2
4
H
4
3
2
1
0
0
2
Death rate
G
3
2
1
0
0
2
Weeks

the rate of instantaneous mortality rises most slowly with time at 23°C and progressively faster at both higher and lower temperatures (fig. 16).

It is also clear from table 3 that the coefficients a (intercept) and b (slope) from the empirical model have their lowest values at 23°C and become progressively larger at both higher and lower temperatures. An attempt has been made to fit a model to these coefficients to provide a descriptive function which enables predictions to be made in two dimensions by the use of a single function.

In the basic survival model

$$\frac{d\,Nt}{dt} = -\mu(t)\,Nt \qquad (10)$$

(Anderson and Whitfield, 1975)

and its solution

$$\mu(t) = a \exp^{bt} \qquad (2)$$

such a single function could be slotted into the models giving

$$\frac{d\,Nt}{dt} = -\mu(t,T)\,Nt \qquad (11)$$

and its solution

$$\mu(t) = a\,(T) \exp^{b(T)t} \qquad (12)$$

where t is time

T is temperature

A second order polynomial model was fitted to each set of coefficients. This model is purely empirical in nature having no underlying biological relevence. It was used because of the diversity of curves that polynomials can produce. The model was of the form

$$c\,(T) = \alpha + \beta T + \gamma T^2 \qquad (13)$$

where c is the dependent variable

T is the independent variable

α, β, γ are empirically determined coefficients.

Figs. 17 and 18 show the calculated polynomial curves,

FIG. 16.

Fig. 16

The variances of the proportion of the population of flukes surviving at a series of consecutive points in time.

1. The solid circles represent the observed variances
2. The solid line shows the fit of the stochastic model (equation 9) to the observed data

A. 17°C

B. 19°C

C. 21°C

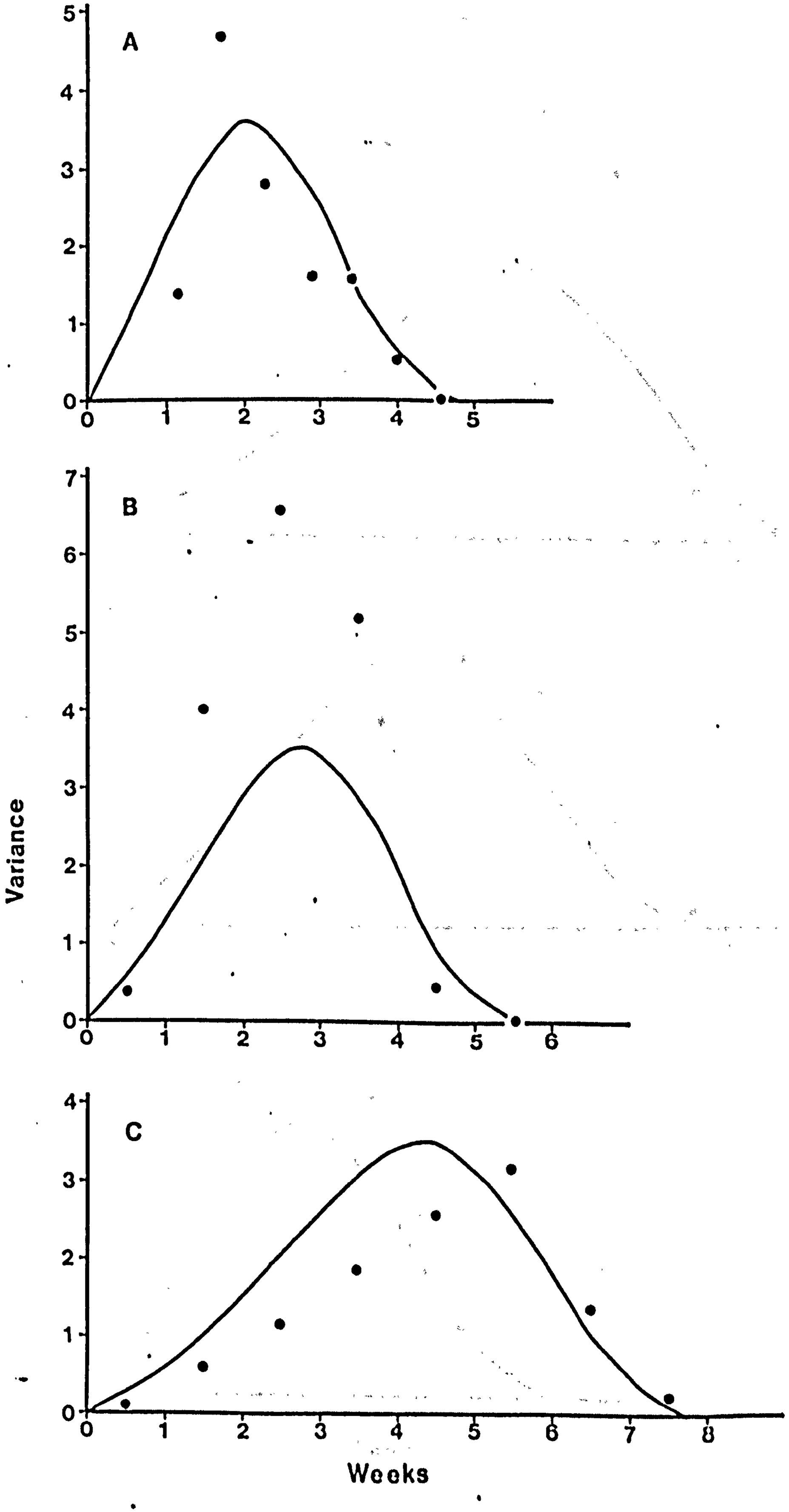
D. 23°C

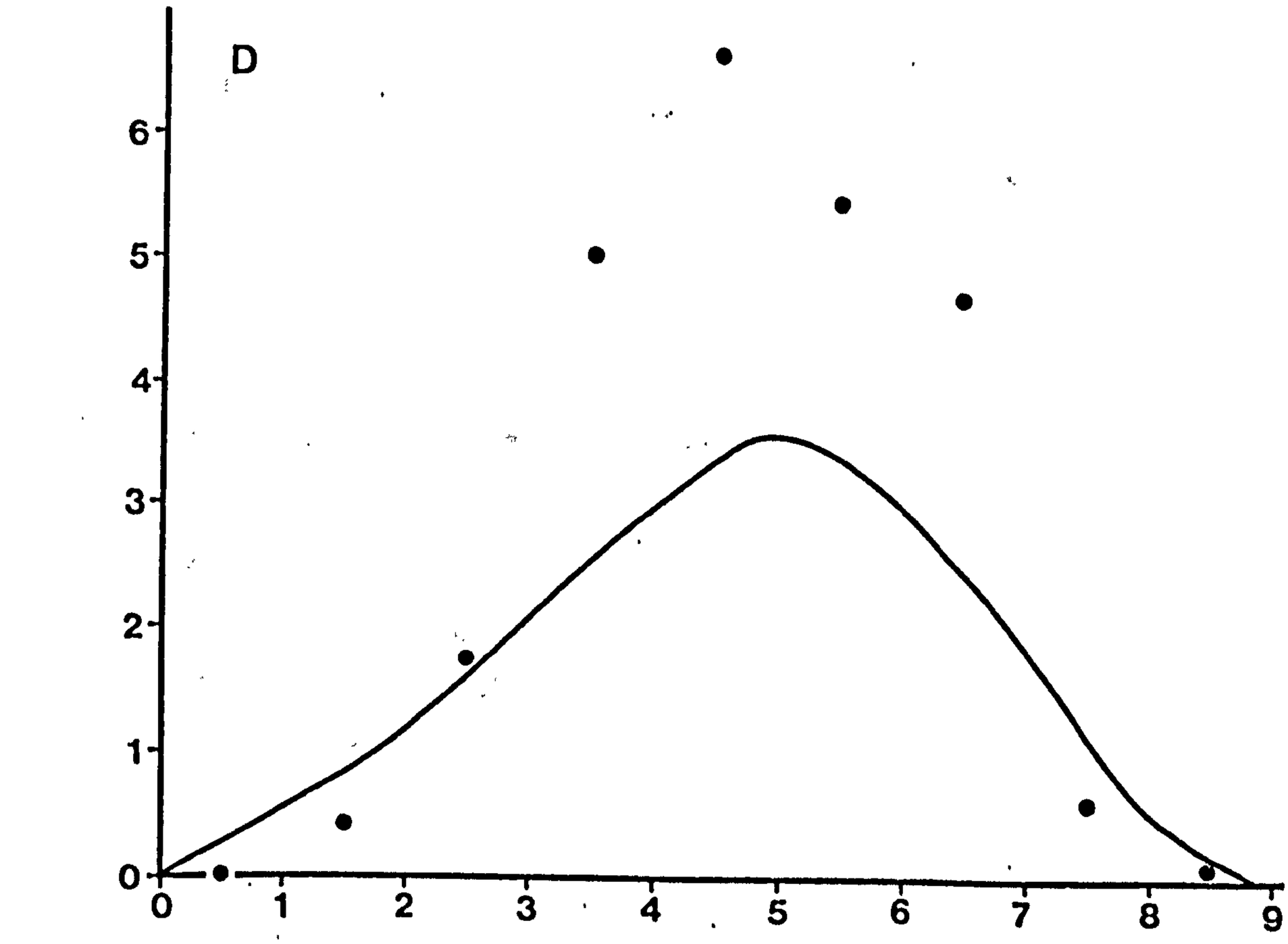
E. 26°C

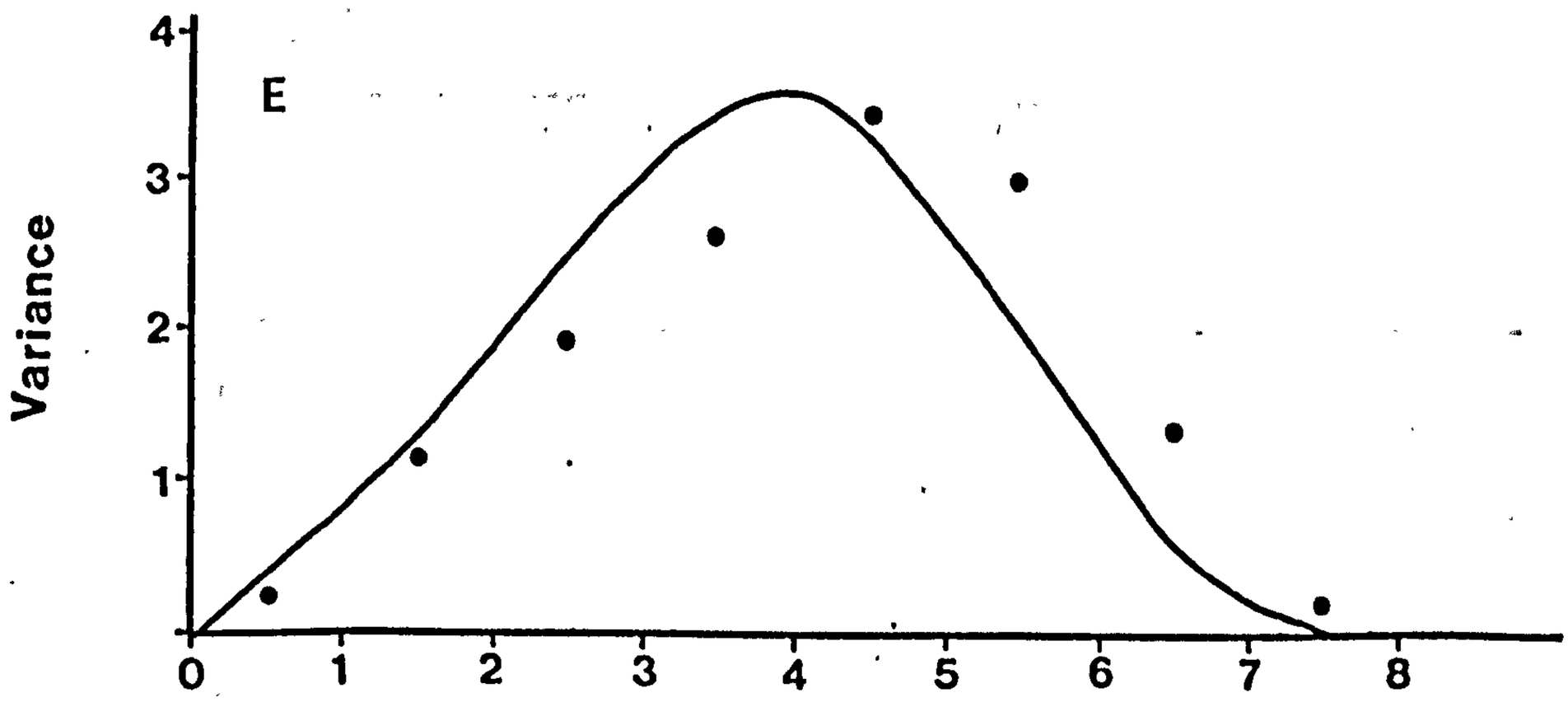
F. 29°C

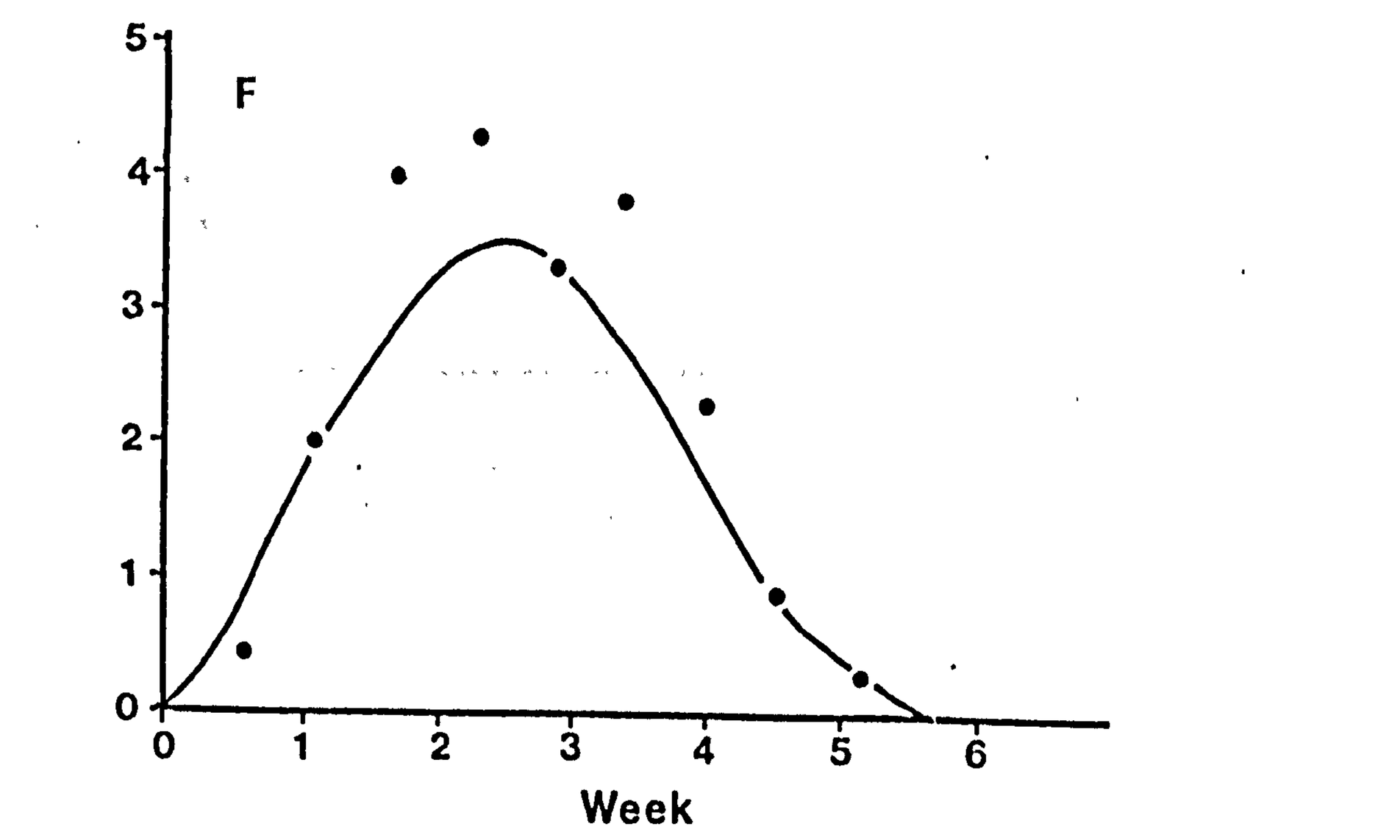
G. 32°C

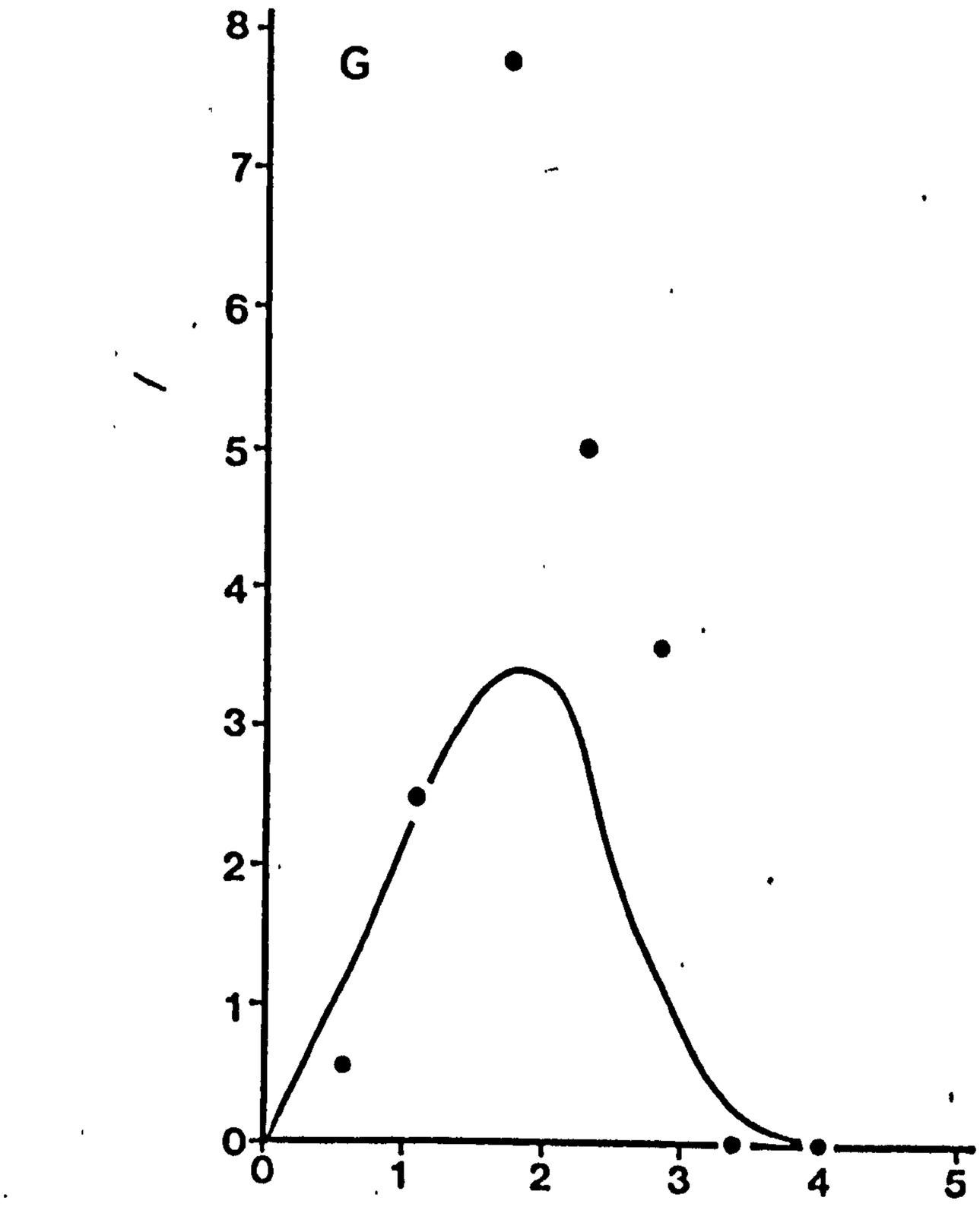
H. 35°C


A
B
C
Variance
Weeks


D
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7 8 9


E
Variance
4
3
2
1
0
0 1 2 3 4 5 6 7 8


F
5
4
3
2
1
0
0 1 2 3 4 5 6
Week


G
H
Variance
Weeks
0
1
2
3
4
5
6
7
8

FIGS. 17, 18.

Fig. 17

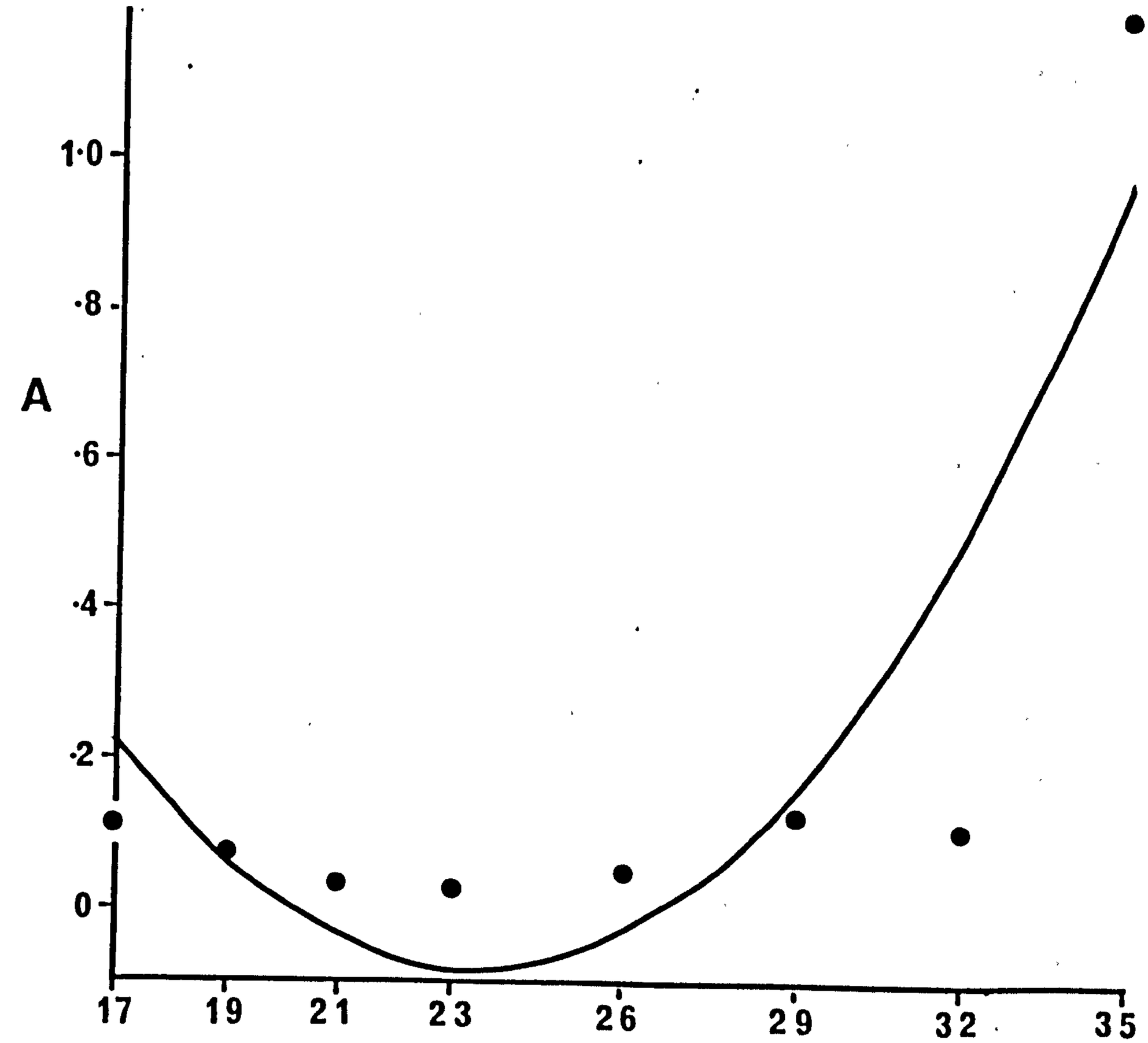
Coefficient A (a) from the empirical model for mortality (equation 2) for a range of temperatures.

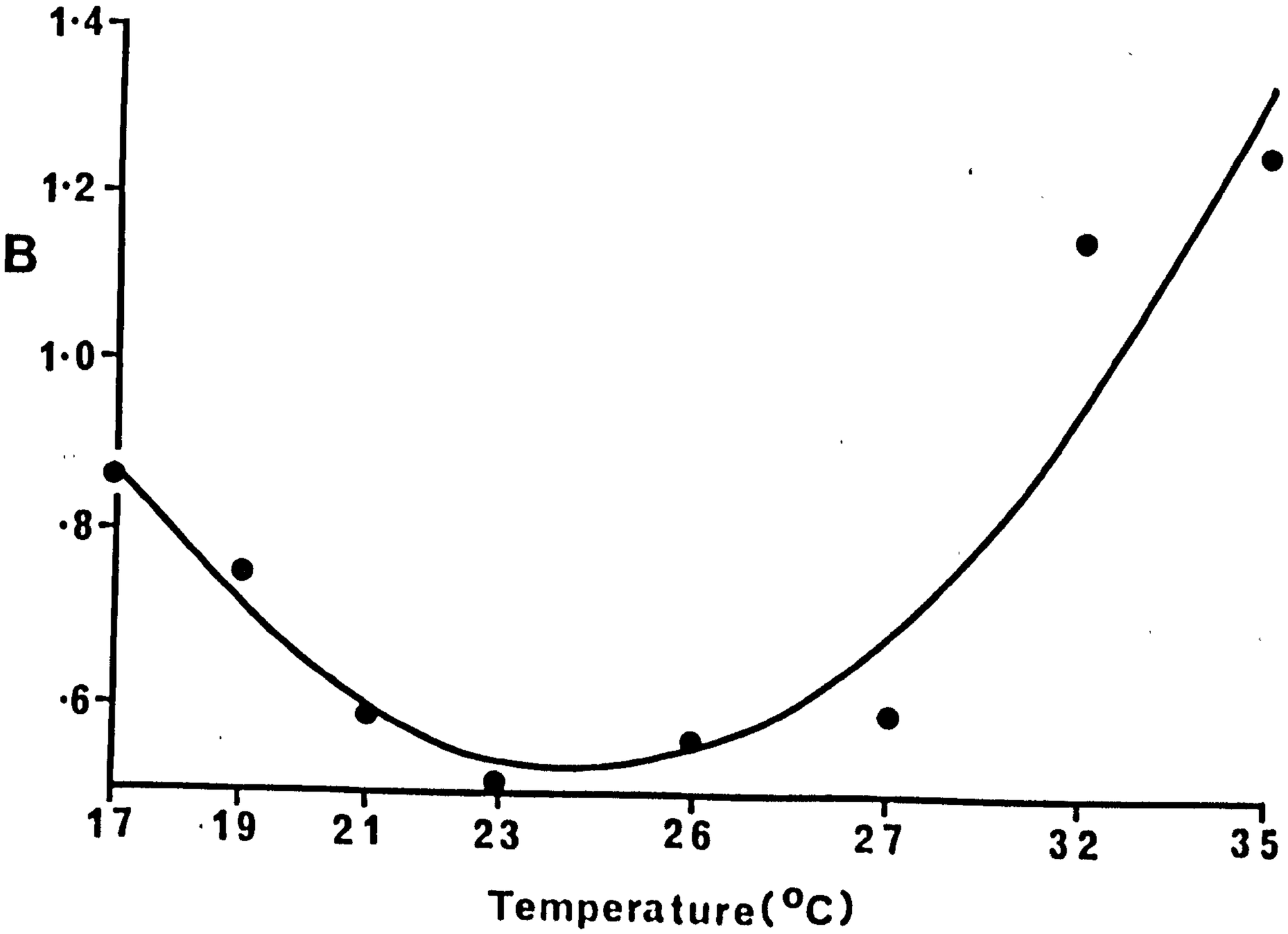
1. The solid circles are the observed values
2. The solid line shows the fit of an empirical second order polynomial model (equation 13)

Fig. 18

Coefficient B (b) from the empirical model for mortality for a range of temperatures against temperature.

1. The solid circles are the observed values
2. The solid line shows the fit of an empirical second order polynomial model (equation 13)


A
1·0
·8
·6
·4
·2
0
17
19
21
23
26
29
32
35


B
1·4
1·2
1·0
·8
·6
17
19
21
23
26
27
32
35
Temperature(°C)

together with the observed data for coefficients a and b, from the survival model. The values of the polynomial coefficients, and the significance of their fit, to the observed data are given in table 14. It is obvious from fig. 17 and table 4, that although the fit of the curve to the intercepts is significant, ($P>0.5$) it is not a particularly satisfactory model, due, in part, to the massive increase in the intercept at 35°C. The significance of this interesting discontinuity will be discussed later. The fit of the model to the slopes is highly significant ($P>.01$), and provides good estimates of the values obtained using the survival model (fig. 18, table 4).

The proportion of flukes surviving at each temperature, at a series of consecutive points in time, predicted by the model described in chapter 3 (Anderson and Whitfield, 1975), are in close agreement with the observed results (fig. 13, table 5). Meaningful estimates of survival substituting the coefficients predicted by the polynomial model into the survival model were not feasible however. This was due to the poor fit of the polynomial model to the intercepts from the survival model giving, in three cases, negative values.

The observed variances are, on average, considerably greater than those predicted by the stochastic model for estimating variance described in chapter 3 (Anderson and Whitfield, 1975).

b) Fecundity

From the rates of egg production per surviving fluke, is is obvious that egg production is highly temperature dependent (Figs. 19, 20, table 11). No egg production was observed at the extreme ends of the temperature range used (17-35°C).

From 19-29°C egg production per surviving fluke rose progressively faster, with time, to a higher peak. At 32°C, although the

FIG. 19.

Fig. 19

Egg production per surviving fluke per hour, against temperature, with an initial parasite density of 14 flukes per host.

A. 19°C

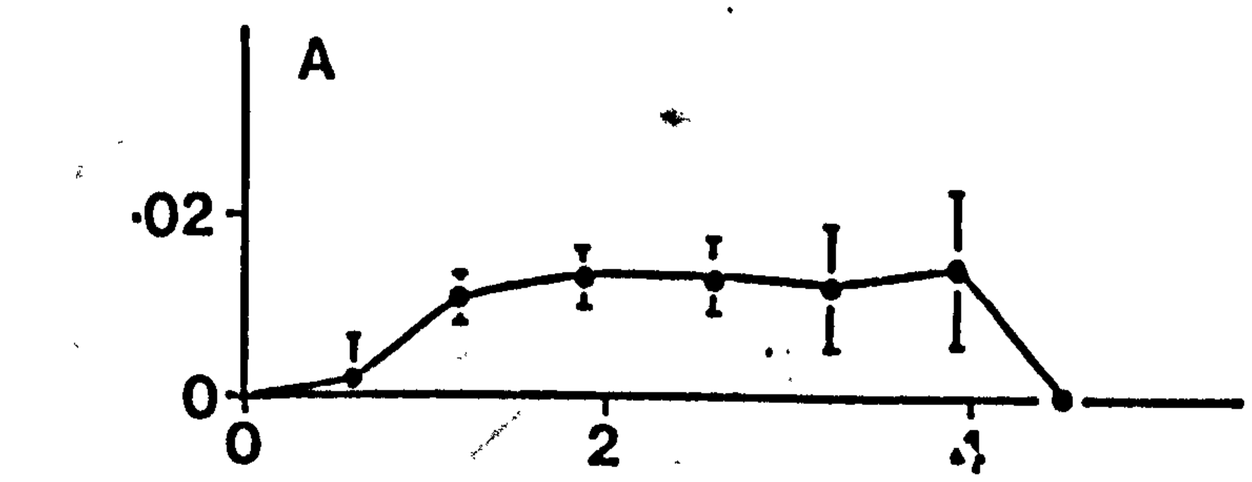
B. 21°C

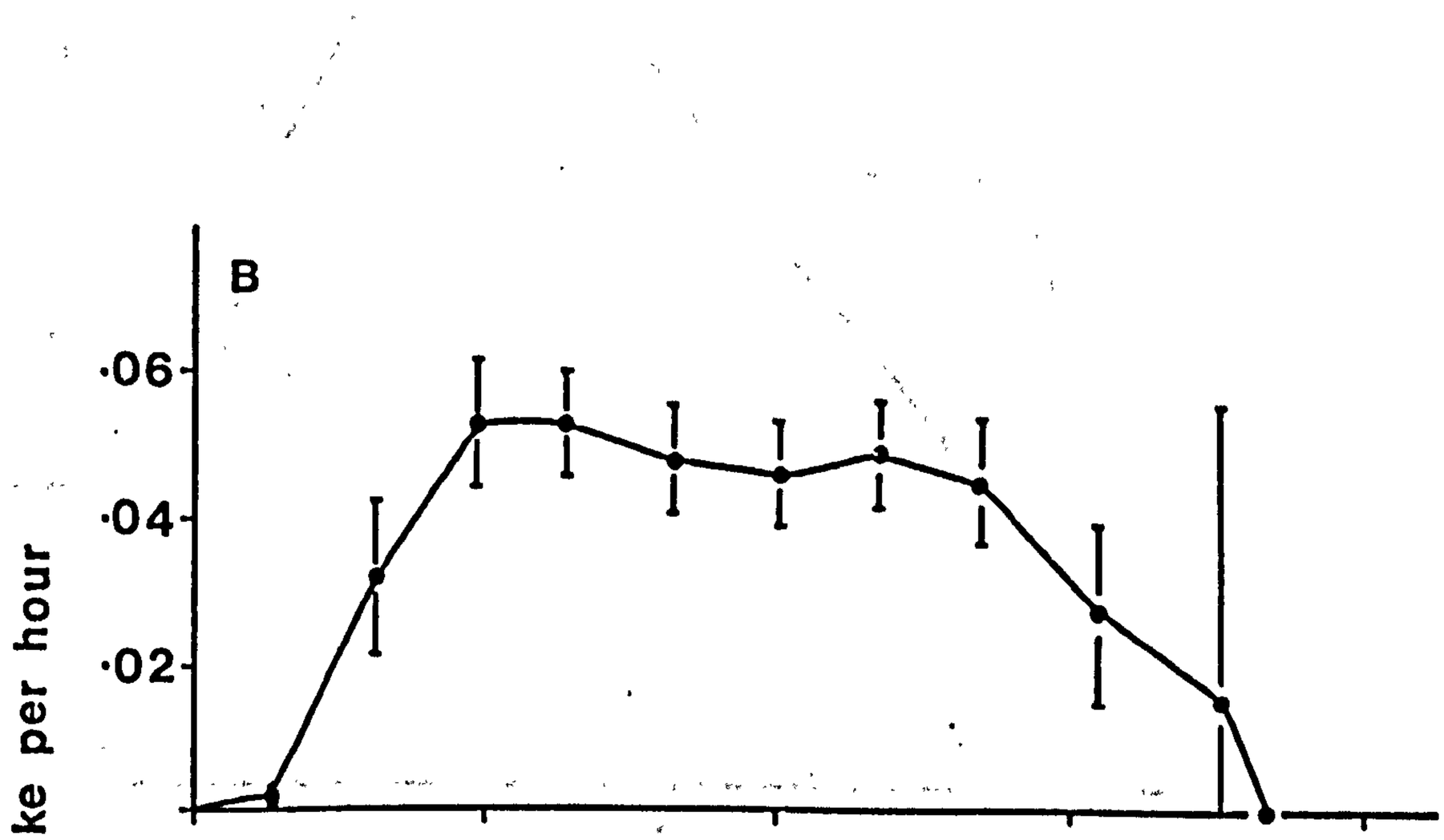
C. 23°C

D. 26°C

E. 29°C

F. 32°C


A
·02
0
0
2


B
·06
·04
·02

Eggs per fluke per hour
C
·12
·10
·08
·06
·04
·02
0
0
2
4
6
8
Weeks

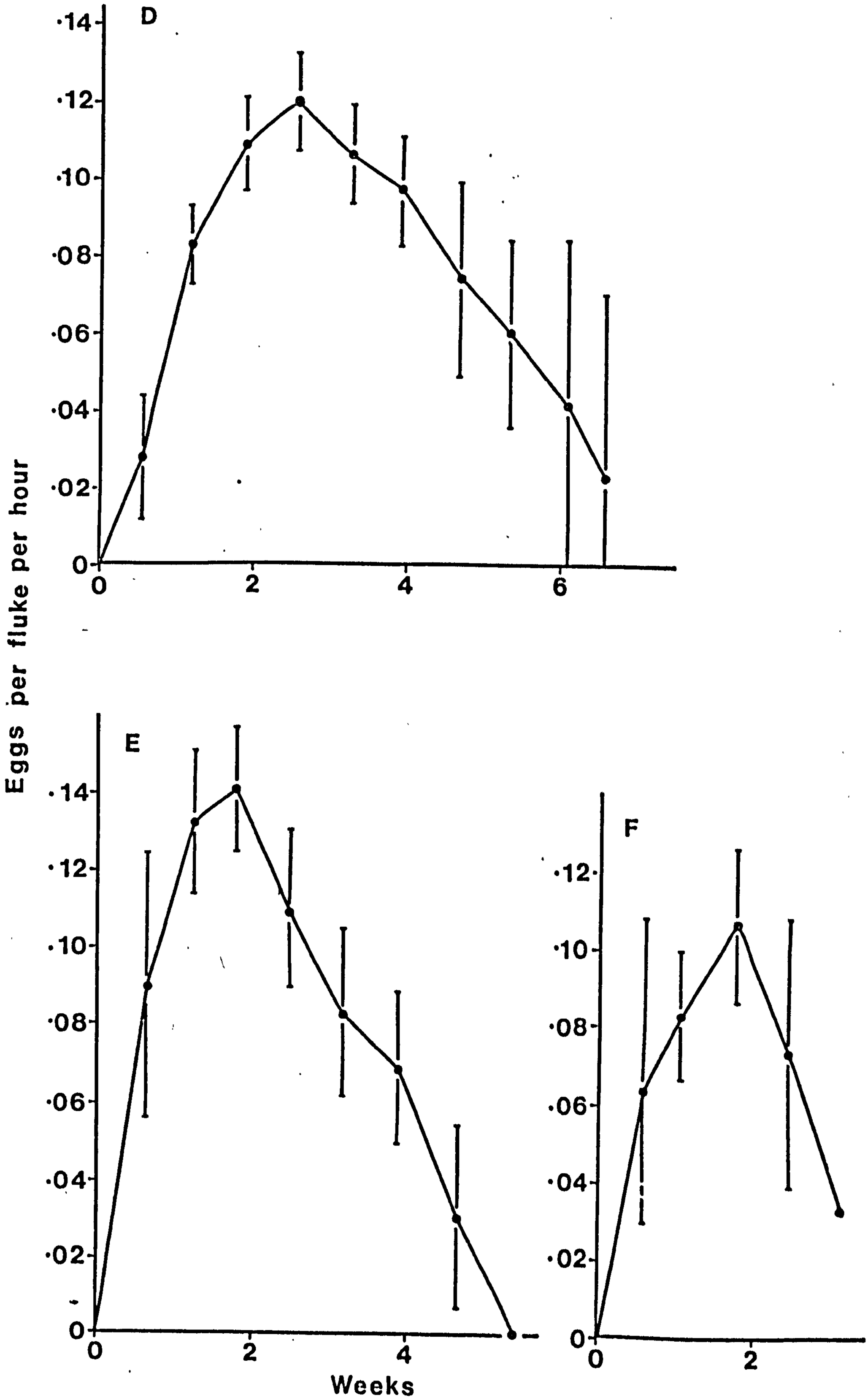
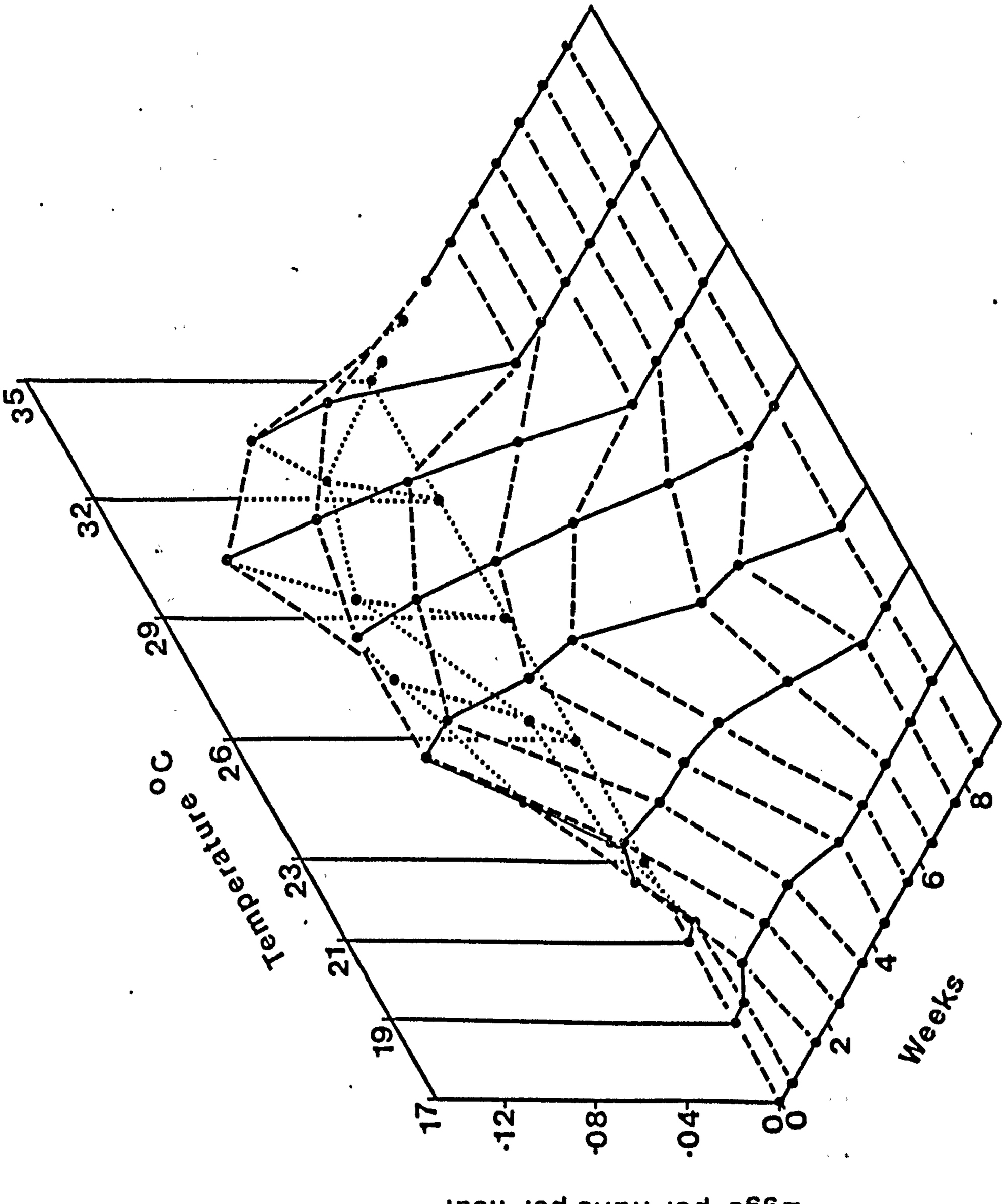

D
·14
·12
·10
·08
·06
·04
·02
0
0
2
4
6
Eggs per fluke per hour
E
·14
·12
·10
·08
·06
·04
·02
0
0
2
4
F
·12
·10
·08
·06
·04
·02
0
0
2
Weeks

FIG.20.

Fig. 20

Egg poduction per surviving parasite per hour, at the mid-points of successive weeks post infection, for six different temperatures.


Eggs per fluke per hour
0
.04
.08
.12
17
19
21
23
26
29
32
35
°C Temperature
2
4
6
8
Weeks

rate of egg production starts to rise faster than at 29°C, the peak output achieved is considerably lower. From 19-23°C the span of egg production increased, due to the increasing life-span of the flukes up to 23°C. Above 23°C the temporal span of egg production contracted, due to the descreasing maximum life-span of the flukes. At 19 and 21°C there is a relatively long period over which the maximum rate of egg output at each temperature is maintained. From 23-32°C the peak rate is maintained for a progressively shorter period, which is followed by a steady decline in the average rate of egg production per individual surviving fluke. The rate of decline from peak egg production becomes increasingly steep above 23°C.

An empirical second order polynomial model of the form

$$e(t) = \alpha + \beta t + \gamma t^2 \qquad (14)$$

where e is the dependent variable (egg production)

t is the independent variable (temperature)

α, β, γ are empirically determined constants

was fitted to the observed data for the rate of egg production per surviving fluke at each temperature (fig. 21). Once again this model was used for its generality, there being no particular justification for it in biological terms. The model fitted well to the observed data between 21 and 32°C and slightly less well at 19°C. The values of the polynomial coefficients and the significance of the fits of the polynomial curves are given in table 6.

The mean egg production per surviving fluke, at the midpoints of successive weeks at each temperature, was determined from the graphs in fig. 17 (table 9). By multiplying this egg production per surviving fluke, at the midpoints of successive weeks by the observed proportion of flukes surviving at the midpoints of successive weeks (table 5), a rate of egg production equal to the total output per host in successive weeks divided by the initial number of

FIG.21.

Fig. 21

Egg production per surviving fluke per hour against time.

1. The solid circles represent the observed rate of egg production at a series of consecutive points in time
2. The solid line is the rate predicted by an empirical second order polynomial model (equation 14)

A. 19°C

B. 21°C

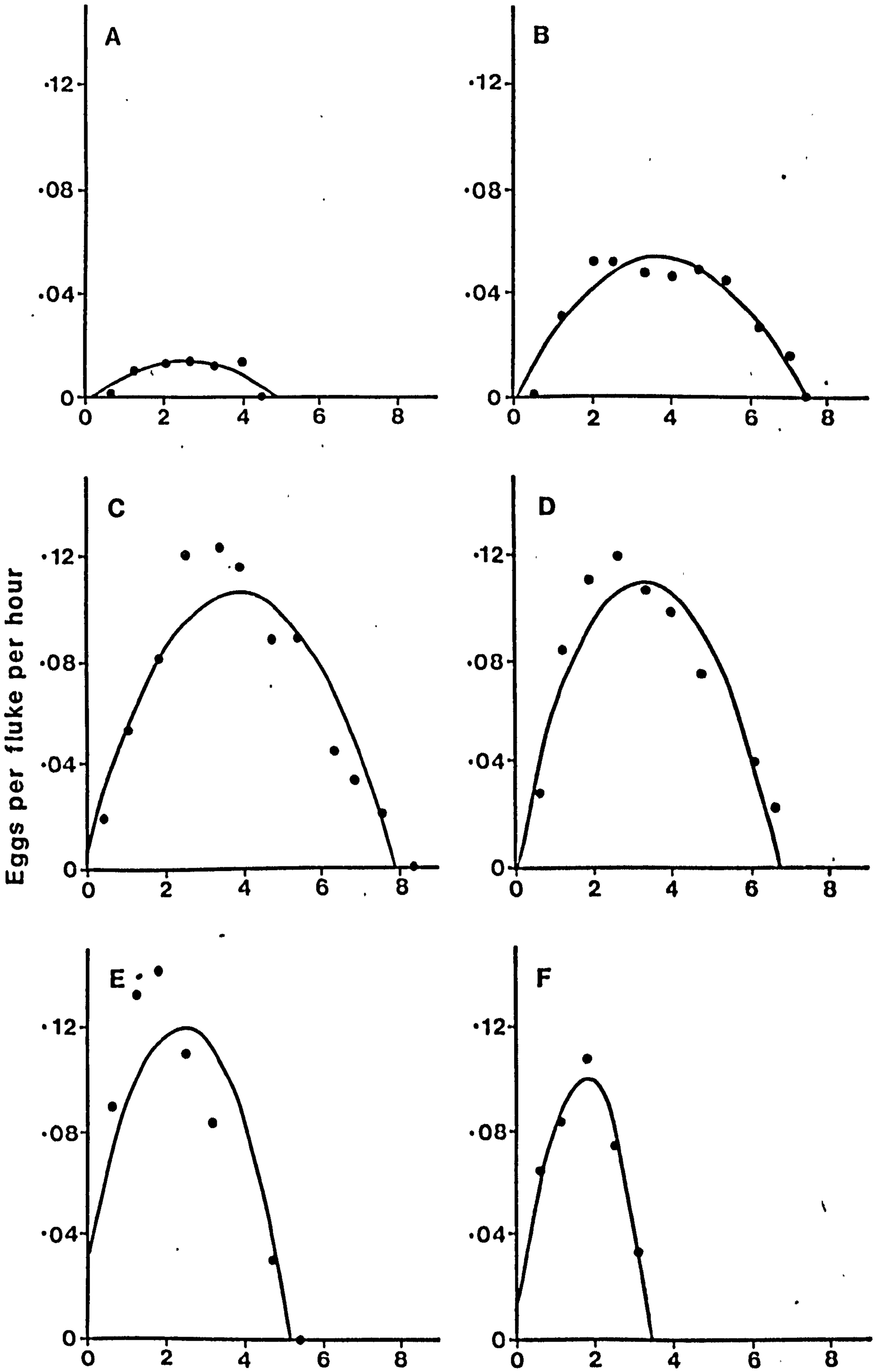
C. 23°C

D. 26°C

E. 29°C

F. 32°C

For the values of the coefficients for the model and the significance of the fit to the observed data see table 6.


A
B
C
D
E
F
·12
·08
·04
0
0
2
4
6
8
Eggs per fluke per hour
Weeks

parasites.

This rate will be termed egg production per average fluke. In this context average refers to the average fluke in the initial population, as distinct from the average surviving fluke.

Whilst this approach might seem complex in the context of this chapter, where the initial fluke density is the same on each host, it provides an essential basis for comparison in chapter 5, where initial parasite density varies. Egg output per average fluke is shown in fig. 22,(table 8).

The interaction between the decrease in survival of flukes and the shorter period of egg production on either side of 23°C results in large drops in total egg production per average fluke above and below 23°C. Cumulative egg production per average fluke during the course of infection is shown in fig. 23 (table 9).

The product of egg production per average fluke, and the initial numbers of parasites, gives the total average egg production per host during the course of infection.

$$\text{i.e. } \lambda\omega = \epsilon \qquad (15)$$

$$\sum_{i=1}^{N} \lambda_i(T)\,\omega_i(T) = \Theta_T \qquad (16)$$

where

λ = egg production per average fluke per week

ω = the initial number of parasites per host

ϵ = average egg production per host per week

Θ = total egg production per host during the course of infection

N = the number of weeks the infection lasts

T = temperature

As in all the experiments in this chapter the initial number of parasites per host was 14

$\lambda_{14} = \epsilon$

FIG.22.

Fig. 22

Egg output per average fluke, per week, at different temperatures.

A. 19°C

B. 21°C

C. 23°C

D. 26°C

E. 29°C

F. 32°C

Egg output per average fluke is the product of the mean egg production per surviving fluke, and the proportion of flukes surviving at the midpoint of each week against time.

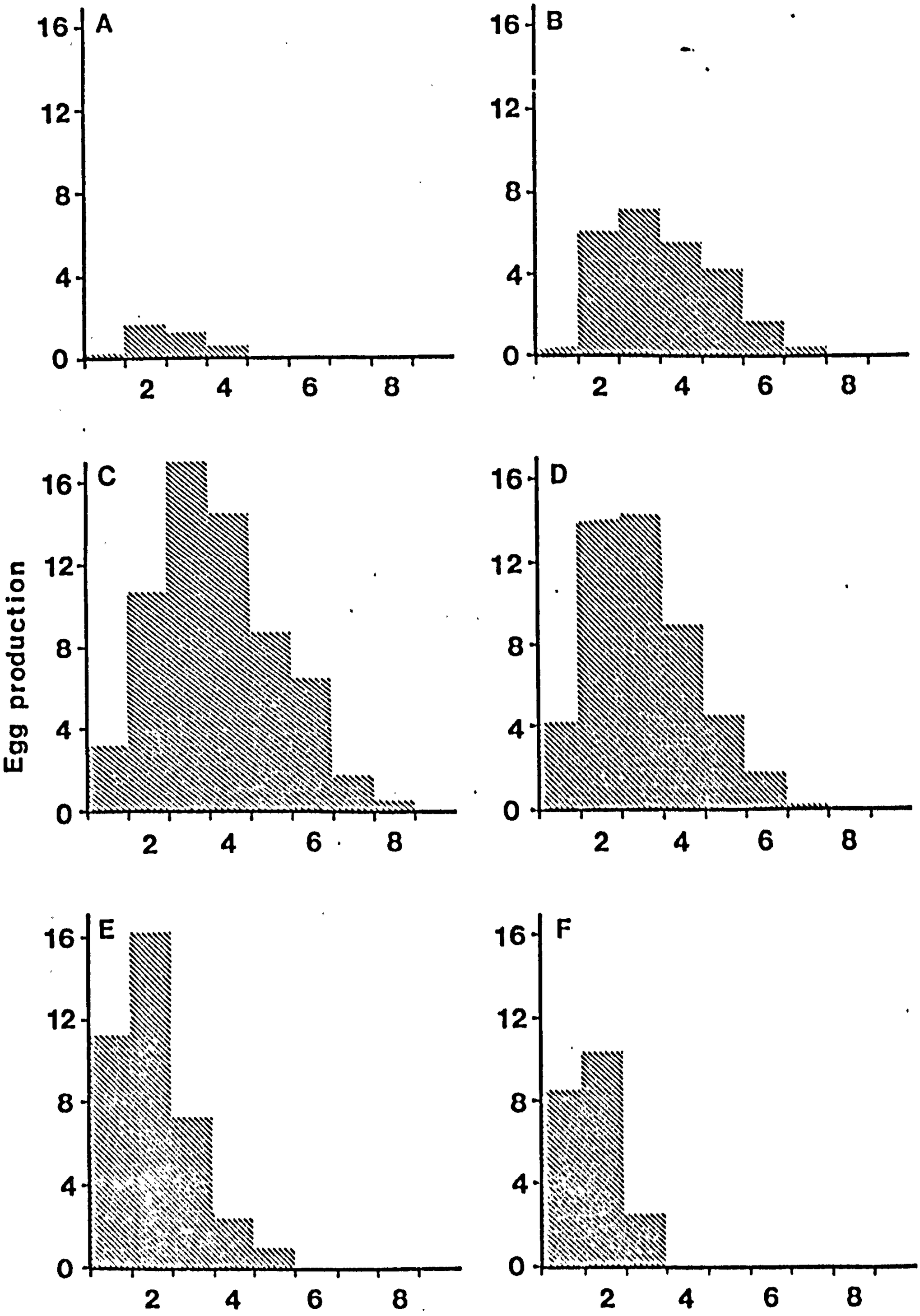

A
B
C
D
E
F
16
12
8
4
0
2
4
6
8
Egg production
Weeks

FIG.23.

Fig. 23

Cumulative egg output per average fluke at different temperatures.

A. 19°C

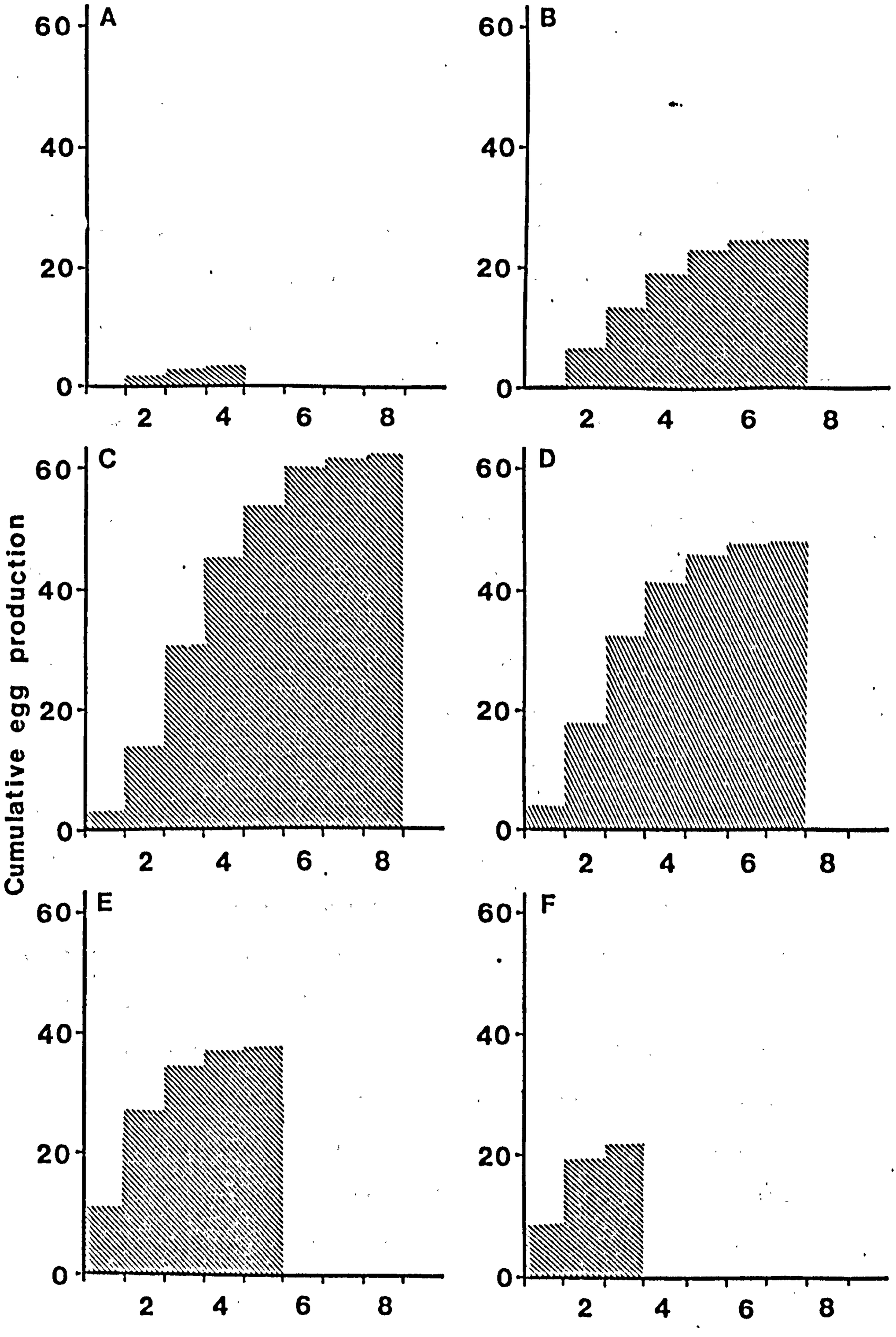
B. 21°C

C. 23°C

D. 26°C

E. 29°C

F. 32°C


A
B
C
D
E
F
60
40
20
0
2
4
6
8
Cumulative egg production
Weeks

and

$$\sum_{i=1}^{N} \lambda_i (T) \quad 14_i = \Theta_T \quad (18)$$

Obviously, in real terms, the processes of survival and egg production discussed here are continuous. The discreet approach adopted here is made necessary by the fare greater complexity involved in dealing with such continuous processes.

As mentioned previously, egg production reflects the interplay of two dynamic processes, survival and egg production per surviving parasite per unit of time, which together give , the egg production per average fluke per unit of time. Fig. 23 shows the cumulative egg output of the average fluke during the course of infection at each temperature. The cumulative total egg production per average fluke is shown in fig. 24 (table 12) and the cumulative total per host (equation 18), at each temperature is displayed in table 13. Fig. 24 shows a steep rise in egg output per infection from zero, at 17°C, to 871 eggs at 23°C, followed by a rather more gentle drop to zero at 35°C. The form of fig. 24 is a result of the complex interplay between survival and the egg production rate of each surviving fluke. Whilst both are temperature dependent they are functionally related to temperature in different ways, giving this complex resultant pattern. For example, the tendency of the rate of egg production per surviving fluke to rise faster with increasing temperature, is offset by a reduction in survival above 23°C. Fig. 22 and table 8 show that egg output per average surviving sluke at 23°C is, however, outstripped during the first week post infection at 26, 29 and 32°C, and for the second week, also at 26 and 29°C. Fig. 23 and table 9 show that the cumulative total for egg output per average fluke per week remains higher than that at 23°C, at 26° and 29° for three weeks post infection and for two at 32°C.

FIG. 24.

Fig. 24

Total cumulative mean egg production by all the parasites per host, during the course of infection, against temperature.

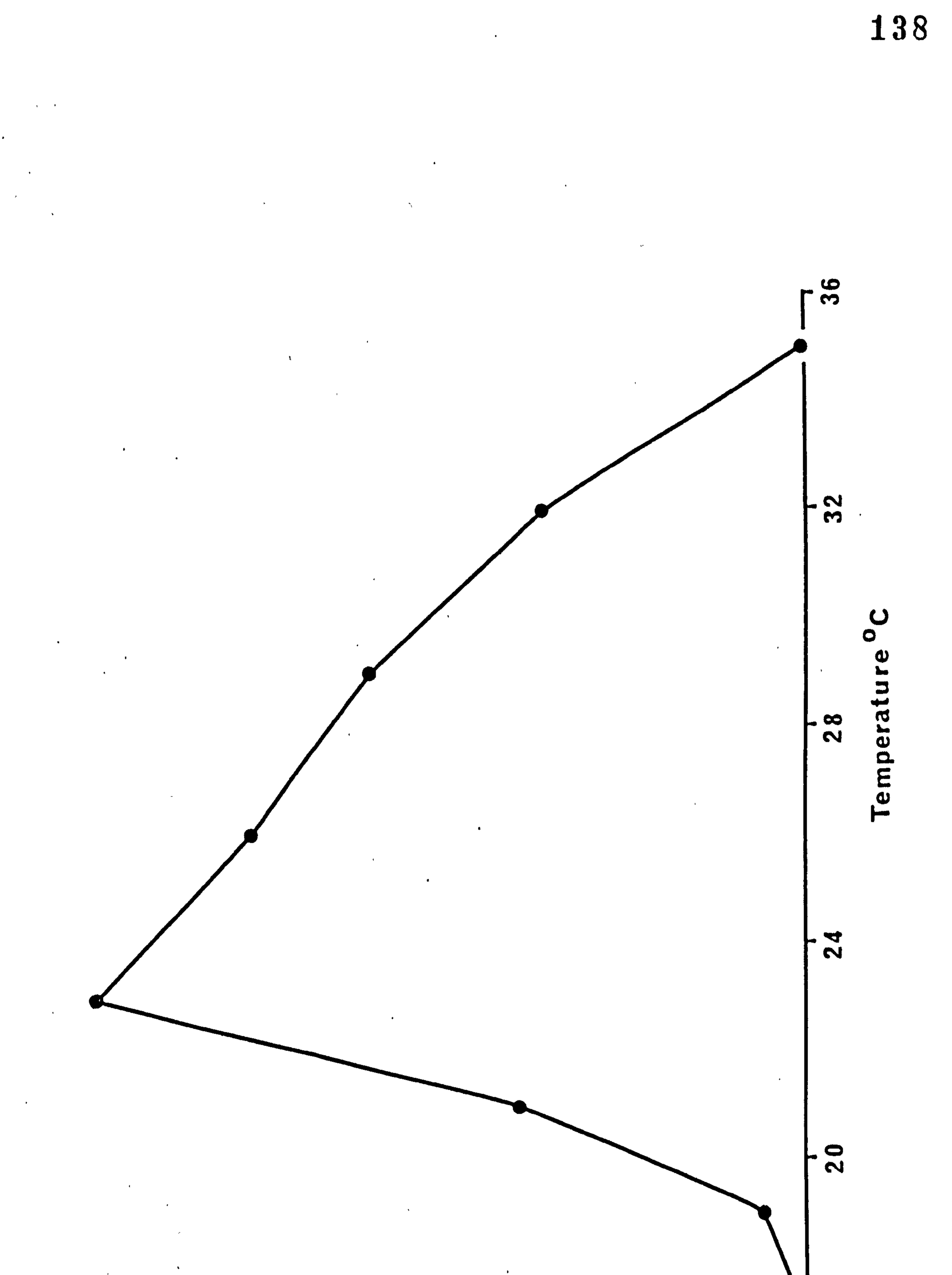

Total egg production
800
600
400
200
0
Temperature °C
16
20
24
28
32
36

Table 3 The values of coefficients a and b and correlation coefficients for the empirical model fitted to the observed instantaneous death rates of parasites at eight differenct temperatures.

Temperature (°C)	17	19	21	23	26	29	32	35
Coefficient a (intercept)	.1009	.0736	.0313	.0280	.04759	.1188	.1061	1.2147
Coefficient b (slope)	.8639	.7571	.5894	.5129	.5598	.5908	1.1509	1.2490
Correlation coefficient r	.9496	.9819	.9804	.9822	.9761	.9457	.9950	.9993
Degrees of freedom	3	4	6	8	6	4	2	0
Level of significance of fit	<.02	<.001	<.001	<.001	<.001	<.01	<.01	-

Table 4

The slopes (B) and intercepts (A) of empirical models (equation 2) fitted to the instantaneous mortality rates of flukes at eight different temperatures with the values predicted by second order polynomial fits to each set of data.

The polynomial coefficients and the significance of the fit of the polynomial curve to the coefficients from the empirical model for instantaneous mortality (equation 2) are also given.

Temperature		Coefficient A		Coefficient B	
		empirical model	polynomial value	empirical model	polynomial value
17		.1009	.2214	.8639	.8750
19		.0736	.0555	.7571	.7104
21		.0313	-.0483	.5894	.5997
23		.0280	-.0899	.5129	.5430
26		.0476	-.0356	.5598	.5596
29		.1188	.1586	.5904	.6963
32		.1061	.4928	1.1509	.9550
35		1.2150	.9669	1.2490	1.3349
Polynomial coefficents	α (intercept)		4.14329		4.45099
	β		-.36287		-.32493
	γ		.00777		.00674
Significance of fit			<.05		<.01

Table 5 The mean proportion of parasites surviving at a series of consecutive points in time. Observed values and values calculated from an empirical model (equation 4).

Temperature °C	17		19		21		23		26		29		32		35	
	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
Weekly Midpoints																
0.5	.965	.939	.969	.965	.988	.982	.990	.984	.983	.973	.961	.933	.941	.931	.436	.430
1.5	.655	.733	.815	.814	.928	.927	.940	.939	.887	.894	.703	.751	.636	.653	.006	.005
2.5	.290	.408	.538	.578	.794	.836	.839	.867	.715	.771	.396	.507	.203	.213		
3.5	.081	.102	.211	.278	.682	.694	.717	.760	.516	.596	.185	.250	.009	.006		
4.5	.006	.004	.041	.059	.505	.496	.547	.610	.340	.379	.073	.070				
5.5			.004	.021	.211	.271	.437	.422	.188	.172	.008	.007				
6.5					.074	.091	.243	.228	.059	.049						
7.5					.016	.013	.065	.082	.004	.004						
8.5							.013	.015								
9.5							.001	.001								
10.5																

Table 6 The values of coefficients α, β and γ and the significance of second order polynomial fits to observed data for the rate of egg production against time at six different temperatures.

Temperature	19	21	23	26	29	32
Coefficient α	-.00189	-.00234	.01504	.00827	.03399	.00153
Coefficient β	.01292	.02998	.04717	.06168	.07170	.11661
Coefficient γ	-.00261	-.00400	-.00615	-.00934	-.01508	-.03422
F. value	9.778	44.590	26.123	36.334	11.186	75.059
Degrees of freedom	2, 5	2, 9	2, 9	2, 8	2, 6	2, 3
Level of significance	$<.05$	$<.01$	$<.01$	$<.01$	$<.01$	$<.01$

Table 7 The rate of egg production per surviving fluke per hour at the midpoints of successive weeks estimated from fig. 19

Temperature	19	21	23	26	29	32
Weekly midpoints						
0.5	.0015	.002	.019	.025	.070	.054
1.5	.012	.039	.067	.094	.137	.097
2.5	.013	.053	.120	.119	.109	.073
3.5	.013	.048	.120	.104	.077	.000
4.5	.000	.048	.095	.080	.040	
5.5		.043	.087	.056	.000	
6.5		.023	.040	.025		
7.5		.000	.034	.000		
8.5			.000			
9.5						

Table 8 Mean egg production per surviving fluke per week times the proportion of flukes surviving at the mid-points of each week (table 7) (equals egg production per average fluke).

Temperature	19	21	23	26	29	32
Weeks						
1	0.24	0.33	3.16	4.13	11.3	8.54
2	1.64	6.08	10.58	14.01	16.19	10.37
3	1.18	7.07	16.91	14.30	7.25	2.48
4	0.46	5.50	14.45	9.02	2.39	
5		4.07	8.73	4.57	.47	
6		1.52	6.39	1.77		
7		0.29	1.63	0.25		
8			0.37			
9						

Table 9 Cumulative mean egg production per surviving fluke per week times the proportion of flukes surviving at the midpoint of each week.

Temperature	19	21	23	26	29	32
Week						
1	0.24	0.33	3.16	4.13	11.30	8.54
2	1.88	6.41	13.74	18.14	27.49	19.23
3	3.06	13.48	30.65	32.44	34.74	21.72
4	3.52	18.98	45.10	41.46	37.13	
5		23.05	53.83	46.03	37.60	
6		24.57	60.22	47.80		
7		24.86	61.85	48.05		
8			62.22			
9						

Table 11A Egg output per fluke per hour at 19°C.

Time interval (days)	Mean time (weeks)	Eggs per surviving fluke per hour	Standard deviation	Number of samples	95% confidence limits
1-5	.60	.0021	.0066	10	.0047
6-10	1.20	.0113	.0058	17	.0030
11-15	1.91	.0133	.0064	17	.0033
16-20	2.59	.0133	.0071	16	.0038
21-25	3.27	.0123	.0121	16	.0065
26-30	3.93	.0137	.0156	12	.0087
31-35	4.50	.0000	-	1	-

Table 11B Egg ouput per fluke per hour at 21°C.

Time interval (days)	Mean time (weeks)	Eggs per surviving fluke per hour	Standard deviation	Number of samples	95% confidence limits
1-5	.55	.0019	.0020	13	.0012
6-10	1.24	.0319	.0182	14	.0105
11-14	1.95	.0533	.0172	18	.0085
16-20	2.54	.0532	.0129	16	.0069
21-25	3.33	.0476	.0132	16	.0069
26-30	4.04	.0464	.0130	16	.0069
31-35	4.69	.0492	.0135	18	.0073
36-40	5.40	.0447	.0158	9	.0079
41-45	6.19	.0270	.0160	4	.0123
46-50	7.04	.0156	.0312	2	.0496
51-55	7.36	.0000	-		-

Table 11C Egg output per fluke per hour at 23°C

Time interval (days)	Mean time (weeks)	Eggs per surviving fluke per hour	Standard deviation	Number of samples	95% confidence limits
1-5	.44	.0189	.0156	6	.0164
6-10	1.04	.0539	.0172	11	.0116
11-15	1.83	.0810	.0278	10	.0199
16-20	2.54	.1198	.0214	7	.0198
21-25	3.34	.1232	.0304	13	.0184
26-30	3.91	.1146	.0228	12	.0145
31-35	4.69	.0865	.0342	12	.0217
36-40	5.46	.0876	.0182	9	.0140
41-45	6.27	.0436	.0167	11	.0112
46-50	6.83	.0330	.0249	8	.0206
51-55	7.59	.0210	.0235	5	.0292
56-60	8.39	.000	-	4	-

Table 11D Egg output per fluke per hour at 26°C.

Time interval (days)	Mean time (weeks)	Eggs per surviving fluke per hour	Standard deviation	Number of samples	95% confidence limits
1-5	.57	.0282	.0277	14	.0160
6-10	1.23	.0834	.0198	17	.0120
11-15	1.92	.1094	.0280	17	.0144
16-20	2.61	.1197	.0231	16	.0124
21-25	3.30	.1065	.0235	16	.0125
26-30	3.95	.0972	.0247	14	.0143
31-35	4.71	.0737	.0326	9	.0250
36-40	5.36	.0598	.0380	12	.0241
41-45	6.11	.0413	.0346	5	.0429
46-50	6.57	.226	.0297	4	.0473

Table 11E Egg output per fluke per hour at 29°C.

Time interval (days)	Mean time (weeks)	Eggs per surviving fluke per hour	Standard deviation	Number of samples	95% confidence limits
1-5	.64	.089	.048	10	.0344
6-10	1.24	.132	.032	14	.0186
11-15	1.82	.141	.018	7	.0166
16-20	2.48	.109	.030	11	.0201
21-25	3.20	.083	.034	12	.0216
26-30	3.88	.069	.021	7	.0193
31-35	4.68	.030	.036	11	.0243
36-40	5.43	.000	-	1	-

Table 11F Egg output per fluke per hour at 32°C.

Time interval (days)	Mean time (weeks)	Eggs per surviving fluke per hour	Standard deviation	Number of samples	95% confidence limits
1-5	.61	.064	.037	7	.0343
6-10	1.08	.083	.026	12	.0165
11-15	1.81	.107	.035	15	.0193
16-20	2.48	.073	.046	9	.0353
21-25	3.14	.033	.0580	3	-

Tables 12 and 13

Temperature (°C)	Table 12 Total cumulative egg production per surviving fluke per week times the proportion of flukes surviving at the midpoint of each week (equals egg production per average fluke).	Table 13 Total cumulative mean egg production by all the flukes per host during the course of infection (table 12 x 14).
19	3.52	49.28
21	24.86	348.04
23	62.22	871.08
26	48.05	672.7
29	37.6	526.4
32	21.72	304.08

CHAPTER 5

Density Dependent Survival and Fecundity

The experiments described in this section deal with the influence of population density on the two population processes, survival and fecundity. Solomon (1949), in a review of "the natural control of animal populations", defines density-dependent action as "that which intensifies (per individual) as population increases and relaxes as density falls". This results in density dependence being the principal agent of control for animal numbers, although it is added that a few processes are inversely related to density.

A considerable difficulty in the study of effects of density dependence on population processes, such as survival and fecundity, is that these processes are concomitantly age dependent. It is often hard to distinguish the relative effects of these two factors in free living organisms, but the difficulty is particularly intense for many parasitic organisms. This is partly because the regular assessment of population numbers and fecundity often presents severe technical difficulties.

The approach adopted here has been to fit a series of survival curves to the results of a series of experiments using different initial parasite densities. This approach is a compromise which assumes that the density dependent effects are a function of the initial inoculum of parasites per host. Anderson and Michel (In press) have found that the survival of Ostertagia ostertagi is dependent on the size of the initial inoculum of nematodes, rather than on the size of the parasite population at any given time.

a) Survival

The series of experiments using hosts in the 28-32 mm size class at 23°C with a varying initial parasite density, shows that survival is density dependent. The survival curves for initial parasite densities of 1, 2 and 14 flukes per host, are extremely similar (fig.25 A-C, table 23). At 30 flukes per host the proportion surviving is a little lower than at lower densities, particularly after 3.5, 4.5 and 5.5 weeks (fig. 25D, table 23). At an average initial density of 72.4 per host there is a much more distinct difference over the first 8.5 weeks post infection (fig. 25E, table 23) though the maximum lifespans of the parasites are similar. At an average initial density of 145.8 parasites per host, the proportion of parasites surviving at the midpoints of successive weeks falls considerably faster than at 72.4 and the maximum life span is also reduced (fig. 25F, table 23). The survival curves are shown in comparative form in fig. 26.

The mean instantaneous death rate at each density was determined using the methods described in chapter 3 (equation 1). The empirical model used in that chapter was used to describe the relationship between death rate and time at each density (fig. 27 A-F). The coefficients from the model are listed in table 14 together with the correlation coefficients for the fit of the predicted curve to the observed instantaneous death rates. For densities between 1 and 72.4 flukes per host $P<.001$ but at 145.8 flukes per host $P<.1>.05$.

In the first six weeks post infection the observed instantaneous death rate at 72.4 flukes per host is slightly higher each week than at any of the lower densities. At all these densities there is a fairly steady increase in instantaneous mortality each week as seen in chapters 3 and 4. At 145.8 flukes per host,

FIG. 25.

Fig. 25

The mean proportion of flukes surviving at a series of consecutive points in time at 23°C.

1. The solid lines are the survival curves predicted by the survival model (equation 4).
2. The solid circles represent the observed proportions surviving.
3. Where present the short horizontal lines denote the extent of the 95% confidence limits to the observed proportions surviving.

A. Initial parasite density 1 per host

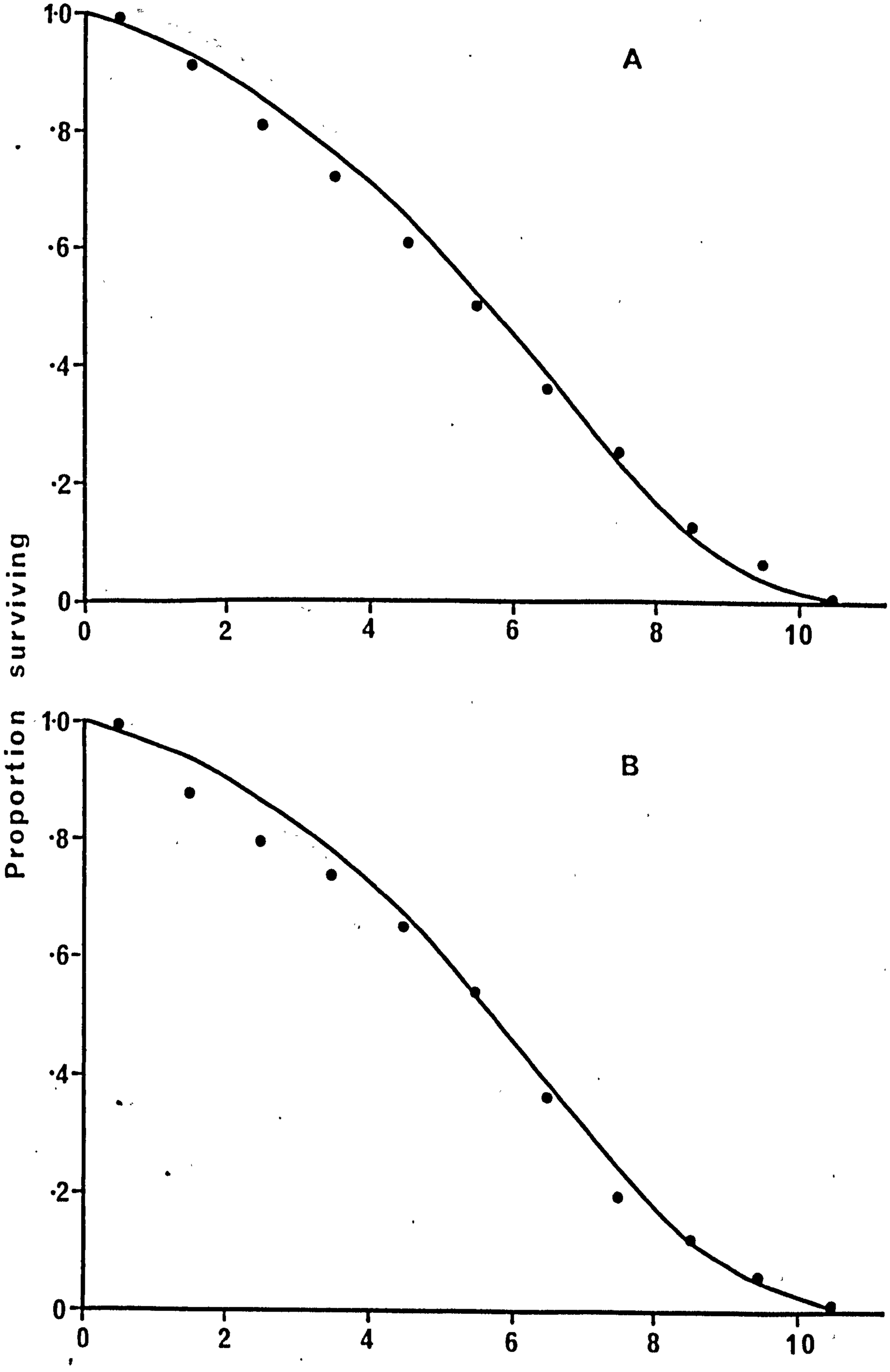
B. Initial parasite density 2 per host

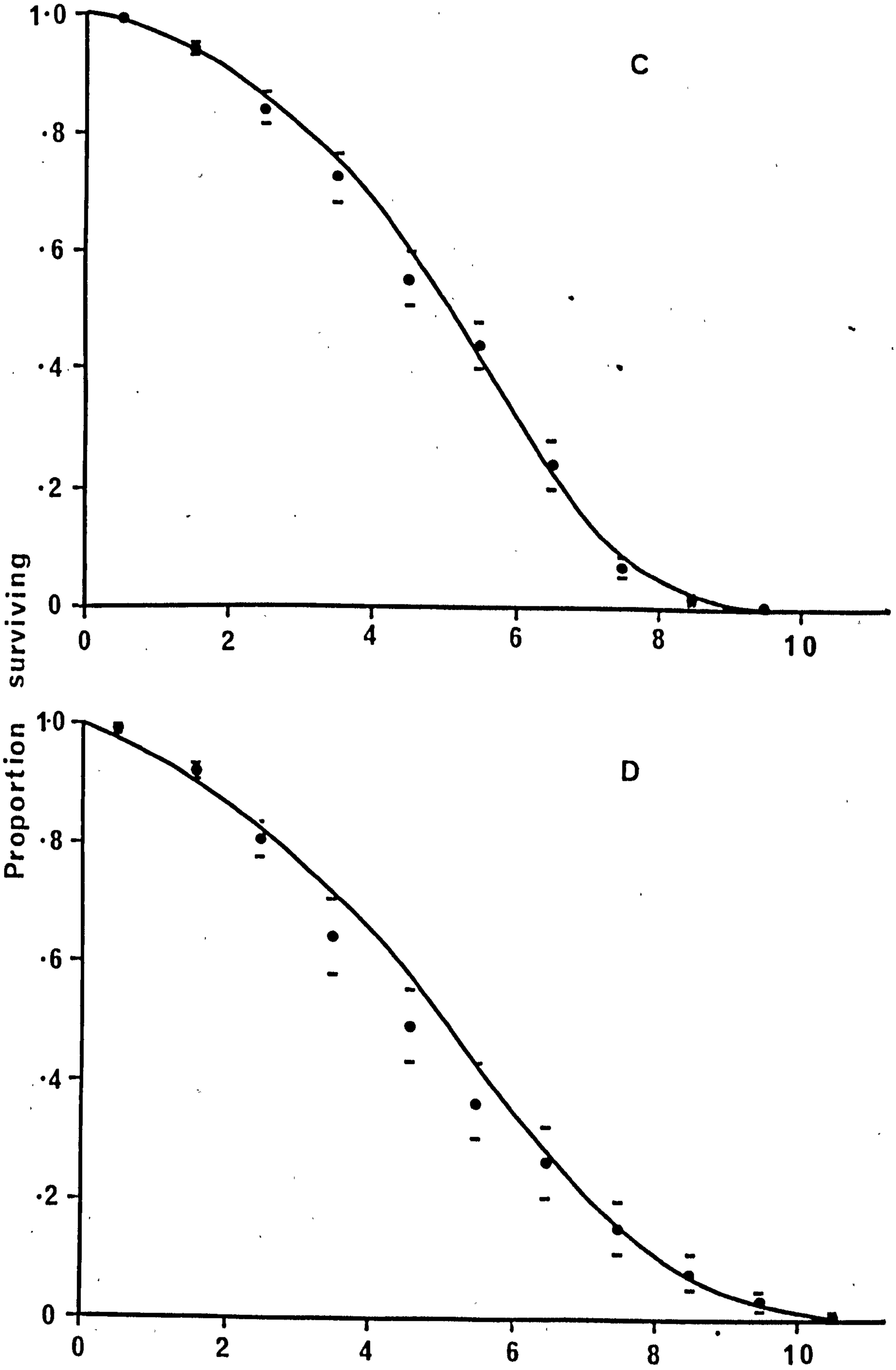
C. Initial parasite density 14 per host

D. Initial parasite density 30 per host

E. Initial parasite density 72 per host

F. Initial parasite density 146 per host


A
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
surviving
Proportion
B
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks


C
D
surviving
Proportion
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks

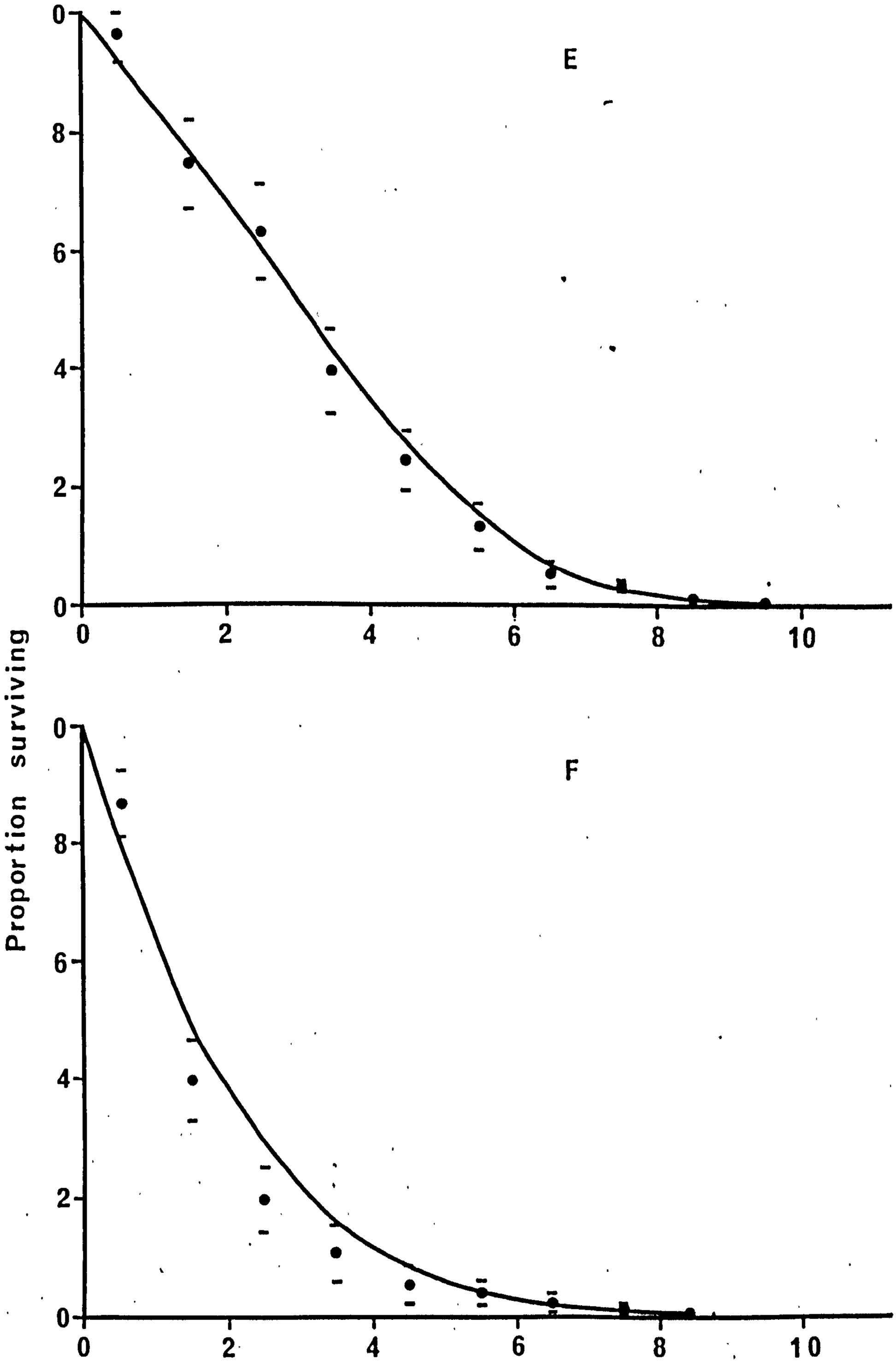

E
F
Proportion surviving
0
8
6
4
2
0
0
2
4
6
8
10
Weeks

FIG.26.

Fig. 26

The proportions of parasites surviving at the midpoints of successive weeks post infection shown for five different initial dens ities of parasite per host.

1. The heavily dashed lines link each time point between the different densities.

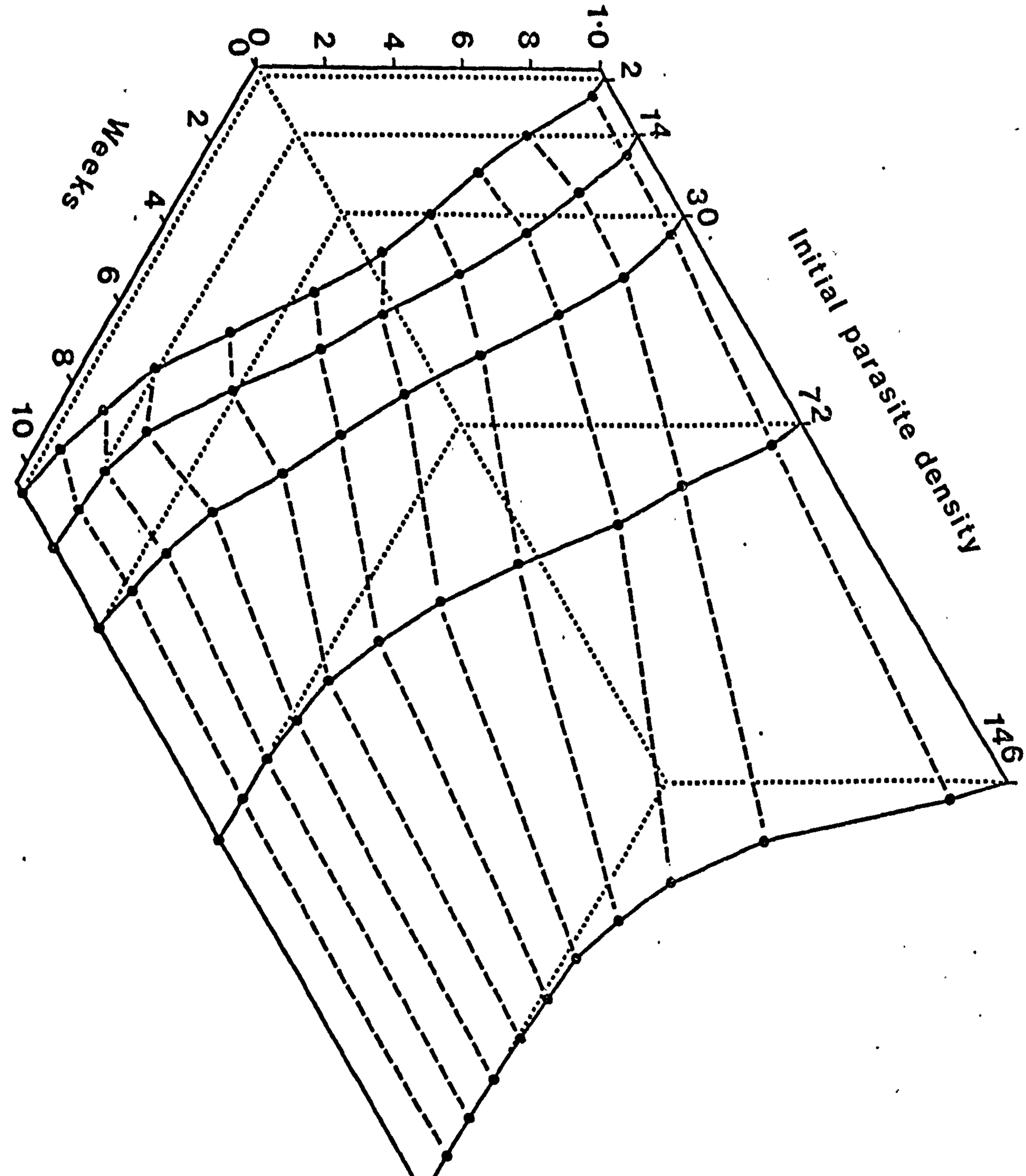

Proportion of flukes surviving
0·0
0·2
0·4
0·6
0·8
1·0
Weeks
2
4
6
8
10
Initial parasite density
2
14
30
72
146

FIG. 27

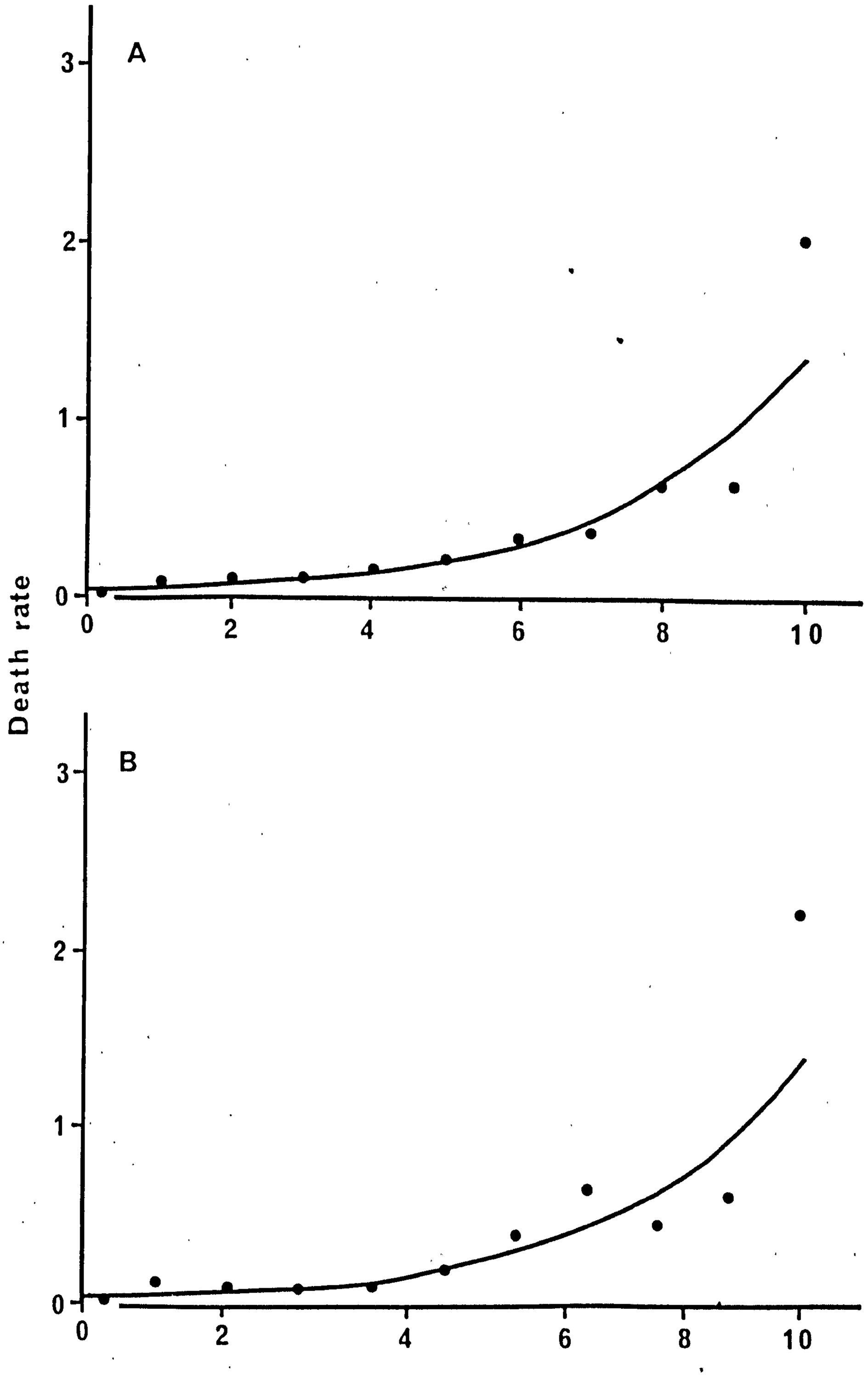
Fig. 27

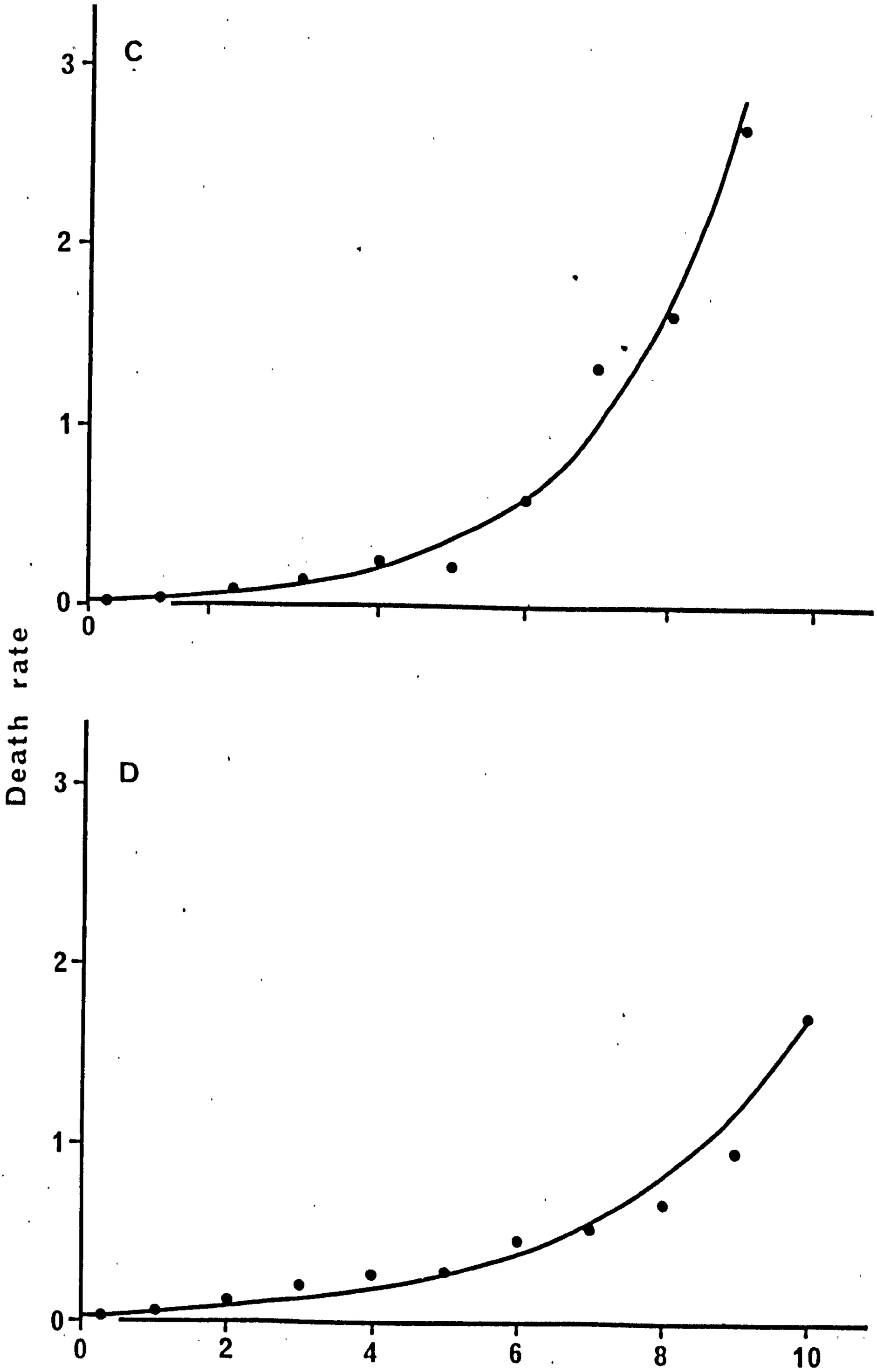
The instantaneous death rates of flukes against time at 23°C with a varying initial parasite density per host.

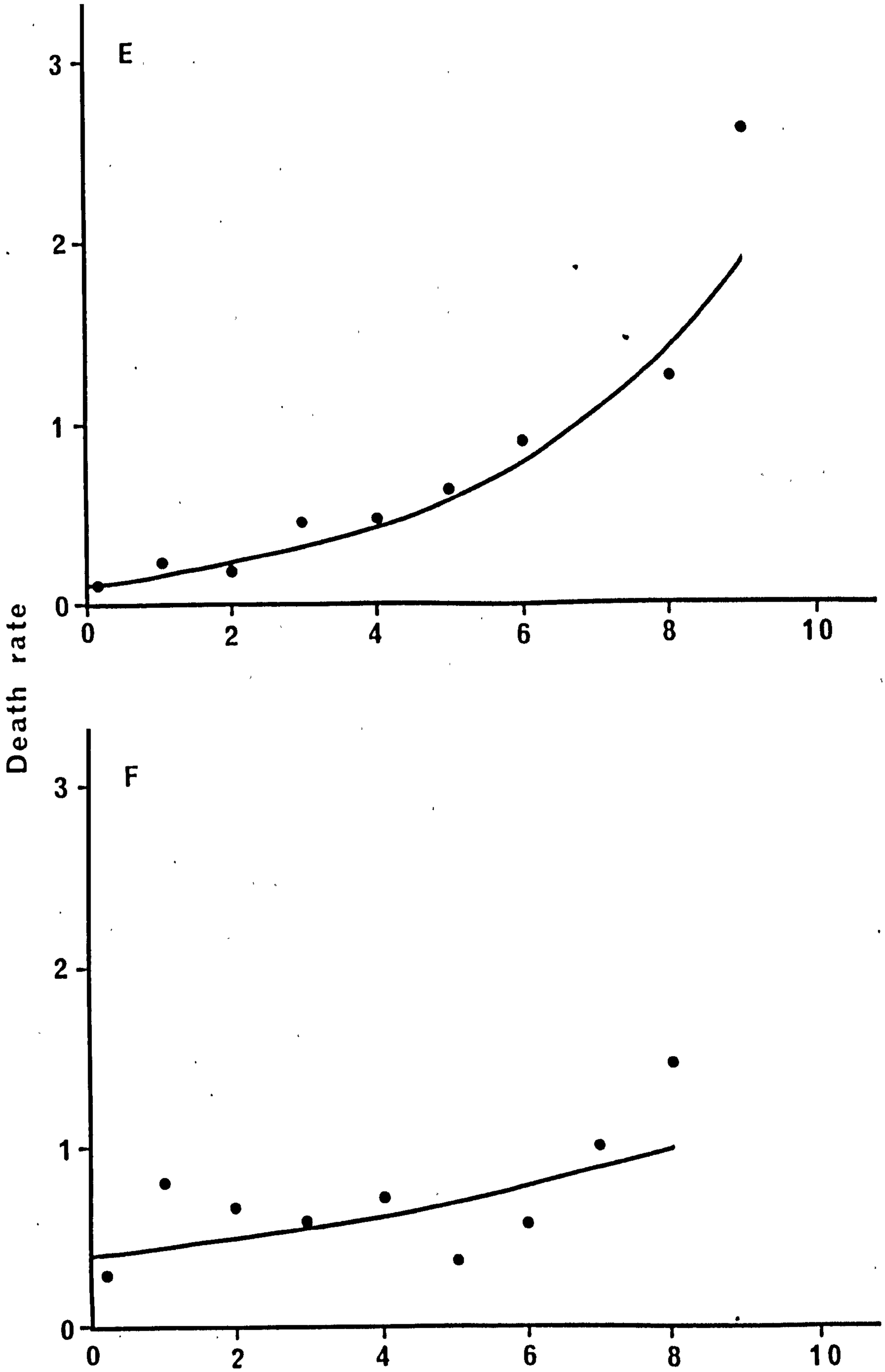
1. The solid circles represent the observed instantaneous death rate (equation 1).
2. The solid line shows the fit of an empirical model (equation 2) to the observed points.

	Initial parasite densities
A.	1
B.	2
C.	14
D.	30
E.	72.4 (average)
F.	145.8 (average)

For the values of the coefficients for equation 2 and the goodness-of-fit of the model to the observed points see table 14.


A
3
2
1
0
0
2
4
6
8
10
Death rate
B
3
2
1
0
0
2
4
6
8
10
Weeks


C
3
2
1
0
0
Death rate
D
3
2
1
0
0
2
4
6
8
10
Weeks


E
3
2
1
0
0
2
4
6
8
10
Death rate
F
3
2
1
0
0
2
4
6
8
10
Weeks

however, the instantaneous death rate is much higher than at 72.4 flukes per host in the first week post infection followed by a huge increase in the second week. After this the death rate remains constant or shows a slight downwards trend until week seven and eight when once more it increases.

An empirical exponential model of the form

$$A(t) = \propto \exp(\beta t) \qquad (19)$$

was fitted to the coefficient a(A) and of the form

$$B(t) = \propto \exp(\beta t) \qquad (20)$$

to coefficient b(B) from the exponential model fitted to the instantaneous death rates for each density class. To increase the number of coefficients for high densities those calculated from the reinfection experiment using hosts originally from the 145.8 density class were used. These reinfected fish formed the 132.9 flukes per host class. This was considered reasonable as there was no evidence that prior infection influenced subsequent infections in any manner whatsoever (chapter 6).

The coefficients derived from the instantaneous mortalities directly using the exponential model and the curves predicted by the second exponential models (equations 19, 20) are shown in figs. 28 and 29. In each case the significance of the fit was good ($P < .005$). Therefore with increasing initial host density the intercept for the exponential empirical model used to describe the change in the instantaneous mortality rate increases in an exponential manner. The slope however (fig. 29) shows a tendency to decrease in an exponential manner with time. If these trends were to continue at densities higher than those used in these experiments the continued increase in the intercept would indicate a situation where a larger and larger proportion of the flukes would die almost immediately following infection and, consequently, the slope would tend closer and

FIGS.28,29.

Fig. 28

Coefficient A (intercept) from the empirical model for mortality (equation 2) for each parasite density against the initial parasite density.

1. The solid circles are the observed values.
2. The solid line shows the fit of an empirical model to the observed points

$$A(t) = \alpha \exp(\beta t) \qquad 19$$

coefficient α = .0288

coefficient β = .0201

r = .9517 P < .005 (seven degrees of freedom)

Fig. 29

Coefficient B(slope) from the empirical model for mortality (equation 2) for each parasite density against initial parasite density.

1. The solid circles are the observed values
2. The solid line shows the fit of an empirical model to the observed points

$$B(t) = \alpha \exp(-\beta t) \qquad 20$$

coefficient α = .4602

coefficient β = - .01055

r = - .9464 P < .005 (seven degrees of freedom)

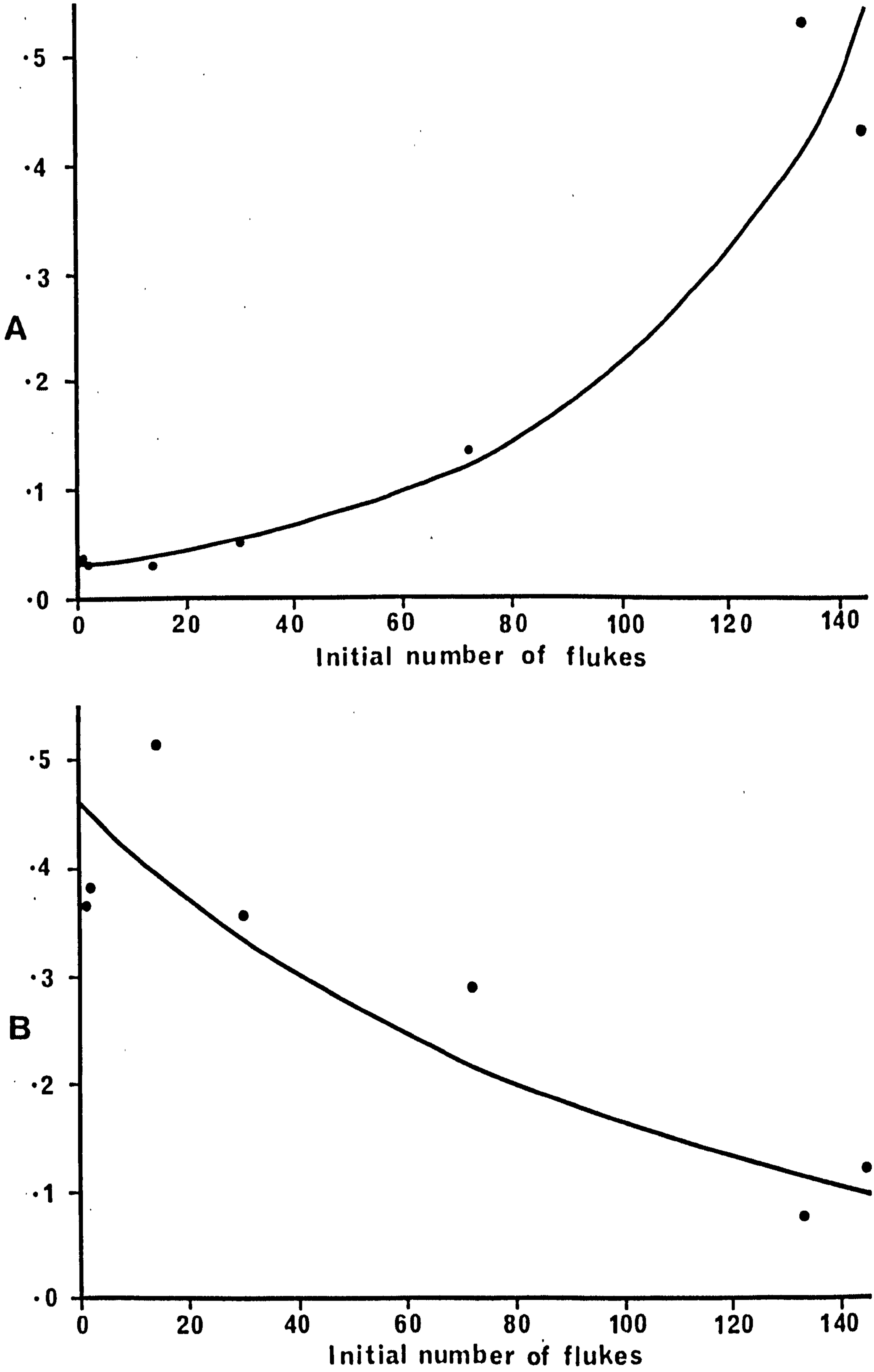

A
·5
·4
·3
·2
·1
·0
0
20
40
60
80
100
120
140
Initial number of flukes
B
·5
·4
·3
·2
·1
·0
0
20
40
60
80
100
120
140
Initial number of flukes

FIG.30.

Fig. 30

Coefficient A (intercept) from the empirical model for mortality (equation 2) for individual heavily infected fish, against the initial number of parasites.

1. The solid circles are the observed values.

2. The solid line shows the fit of an empirical model of the form

$$A(t) = \varepsilon t + \gamma \qquad 21$$

to the observed data.

Coefficient ε = -.2577

Coefficient γ = .00513

r = .6337 $P < .005$ that r is not significant.

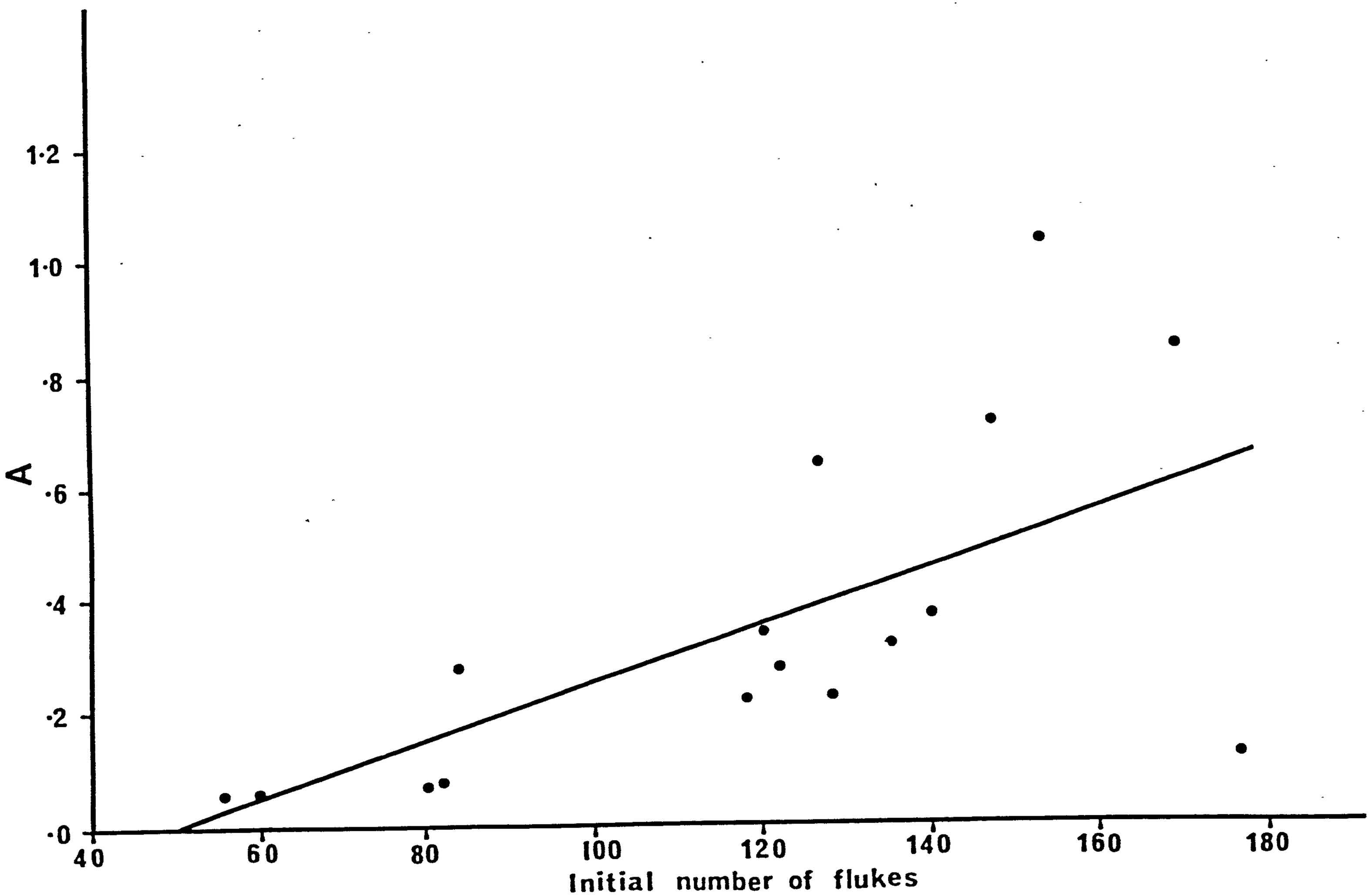

A
1·2
1·0
·8
·6
·4
·2
·0
40
60
80
100
120
140
160
180
Initial number of flukes

FIG.31.

Fig. 31

Coefficient B (slope) from the empirical model for mortality (equation 2) for individual heavily infected fish, against the initial number of parasites.

1. The solid circles are the observed values.

2. The solid line shows the fit of an empirical model of the form,

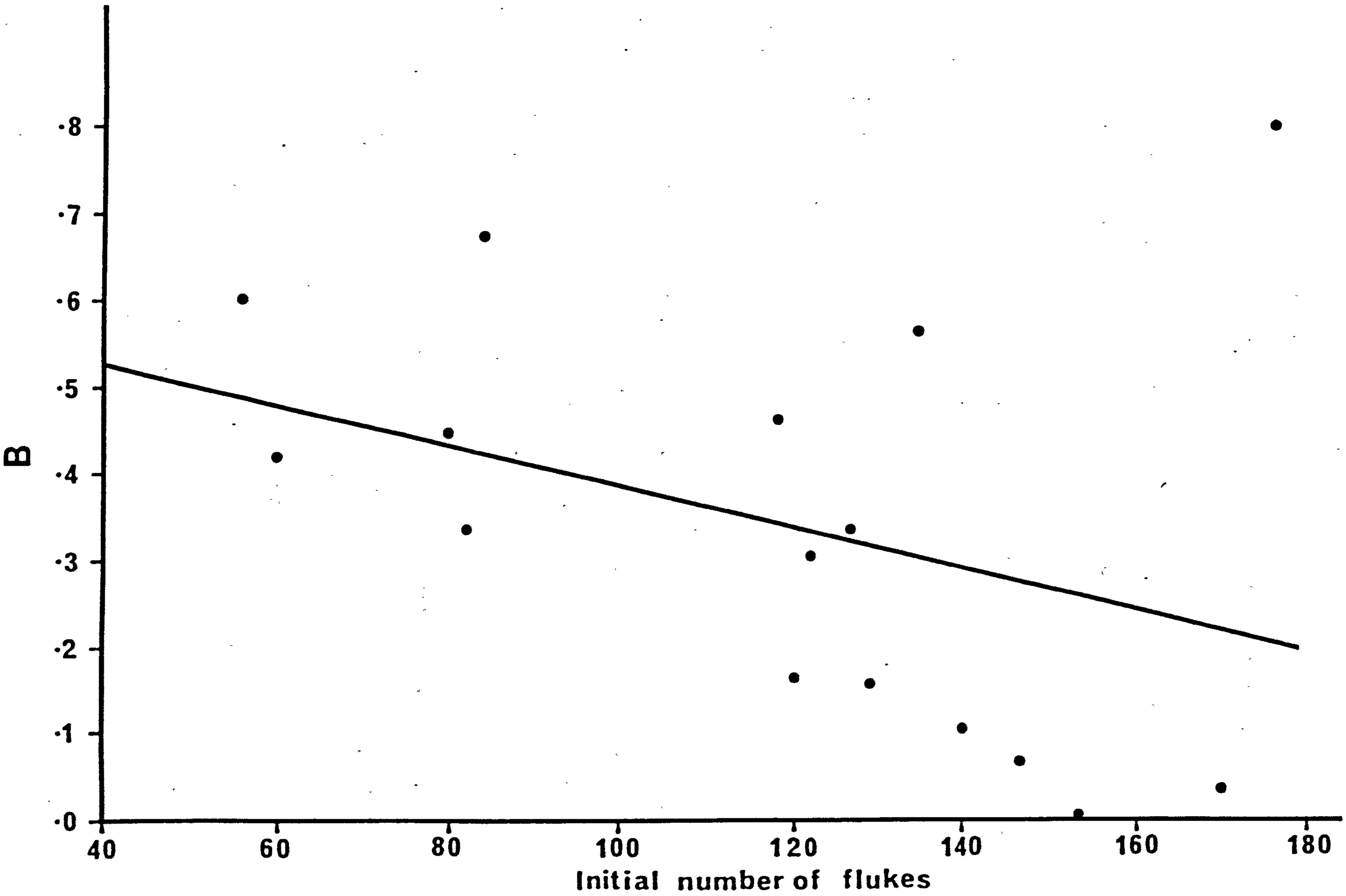
$$B(t) = \xi t + \gamma \qquad 20$$

to the observed data.

Coefficient ξ = .6209

Coefficient γ = -.00235

r = .35966 P <.05 that r is significant.


B
·8
·7
·6
·5
·4
·3
·2
·1
·0
40
60
80
100
120
140
160
180
Initial number of flukes

closer to zero. This is because in biological terms the intercept is the instantaneous death rate at time $0 + \delta t$ where δt is an extremely small time interval.

The new values for the slopes and intercepts predicted for each density class (coefficients a' and b') were determined using the coefficients α and β from the calculated curves shown in figs. 28 and 29. Using coefficients a' and b', curves for instantaneous mortality were determined and are compared with the observed data and the curve predicted using coefficients a and b in table 27. A comparison between the observed proportion of parasites surviving at a series of consecutive points in time and the proportions predicted by coefficients a' and b' is given in table 26 for each density class and can be compared with the proportions predicted by a and b in table 23.

The curves for survival and mortality calculated by the model (equations 2 and 4) using coefficients a' and b' are poorer fits to the observed data than those calculated using a and b. However, the exponential relationships between coefficients a and b and the initial parasite densities and the coefficients a' and b' derived from these relationships do seem to provide a simple model for predicting the approximate overall effect of initial parasite density on mortality.

From the proportions of flukes surviving on each individual heavily infected fish (56-177 flukes per fish) the instantaneous death rates were determined for the flukes on each individual host. Coefficients a and b from equation 2 were then determined for each individual set of data and plotted against the initial number of flukes (tables 14, 15, figs. 30, 31). The intercepts, a(A), showed considerable variation, especially at the higher densities.

A simple linear model of the form,

$$A(t) = \xi t + \gamma \qquad (21)$$

where

ξ is the intercept

γ is the slope

t is time

was fitted to the intercepts from the mortality model (equation 2). This model gave a significant fit to the intercepts ($P < .005$) (fig. 30). The intercepts tended to increase with increasing initial parasite density as would be expected from the grouped data (fig. 28).

The slopes, b(B), were highly variable (fig. 31) but tended to decrease with increasing initial parasite density, again as would be expected from the grouped data (fig. 29). Another simple linear model of the same form as equation 21 was fitted to the calculated slopes

$$B(t) = \xi t + \gamma$$

This model gave, however, a poor fit to the data ($P > .1$) due to the extreme variability of the data.

As mentioned previously, age dependent, and density dependent, processes are acting conjointly. The proportion of deaths attributable to the effects of density have been separated from that attributable to age at the average initial parasite densities of 72.4 and 145.8 parasites per host. This separation has been approached in terms of finite, rather than instantaneous, rates. A finite rate is a simple expression of observed values, for example, in terms of mortality

$$F = \left(1 - \frac{Pt+1}{Pt}\right) \qquad (22)$$

where

Pt is the number of organisms present at time t.

Pt +1 is the number of organisms present at time t + 1

F is the finite rate of change.

An instantaneous rate has a time base that is infinitely small. For example, in terms of mortality

$$\frac{dNt}{dt} = \mu Nt \qquad (23)$$

and $Nt = No\ e^{-\mu t}$ (24)

where

Nt is the population size at time t

No is the population size at timeo

μ is the instantaneous mortality rate derived as follows:

$$\mu = \frac{\ln Nt - \ln Nt+1}{t} \qquad (25)$$

For a fuller discussion of this topic see Krebs (1972).

A finite approach has been adopted here insted of the more rigorous instantaneous methods described previously because the complexity of the interacting processes makes the instantaneous solution extremely difficult. There are as yet no examples of an instantaneous solution to this type of parasitological problem in the literature.

From the observed data (table 23) there is no obvious relationship between initial parasite density and survival between 1 and 30 flukes per host. Also the general model fitted to the observed data using coefficients a′ and b′ derived from equations 19 and 20 only predicts a very small increase in mortality between these initial parasite densities (table 27). Therefore, for the purpose of the following caloulations it is assumed that the mortality curve for the 14 fluke per host initial density class at 23°C is dominated by age dependent factors.

From the proportion of parasites surviving at a series of consecutive points in time at 14 flukes per host (tables 22A, 24A) the chances of a parasite surviving to the next time point is determined. This gives finite death rates for the parasites.

Using the observed proportions of parasites surviving at a series of time points at initial densities of 72.4 and 145.8 flukes per host the expected proportion of flukes surviving to the next time point is determined (tables 22D, 24D). This is done by assuming the chance of survival to the next time point is the same as that for flukes at the 14 fluke per host level (table 22B, 24B).

The actual proportion of flukes surviving to the next point at these high density levels (tables 22C, 24C) is then subtracted from the predicted results (tables 22D, 24D). The result is m_1, the proportion of finite mortality attributable to density each week (tables $22m_1$, $24m_1$). By subtraction of m_1, from M, the total finite death rate (tables $22M$, $24M$) the proportion of the total finite death rate attributable to age dependent factors (m_2) is determined

$$\text{or } M = m_1 + m_2 \qquad (26)$$

The results are displayed in histogram form in fig. 32. Assuming that the effects of finite density dependent mortality are additive the effects of m_1 and m_2 are fairly closely balanced at the initial parasite density level of 72.4 flukes per host. 54.6% of mortality is attributable to m_1 and 45.4% to m_2. Most density dependent mortality occurs in weeks two to four. Generally the relative importance of age dependent mortality tends to increase during the course of infection.

At an initial density of 145.8 flukes per host m_1 predominates with 76.8% of mortality being attributable to density and only 23.2% to age dependent factors. Density dependent mortality is predominant over the first four weeks post infection, especially so in the first two. Age dependent factors again become relatively more important during the course of infection.

FIG.32.

Fig. 32

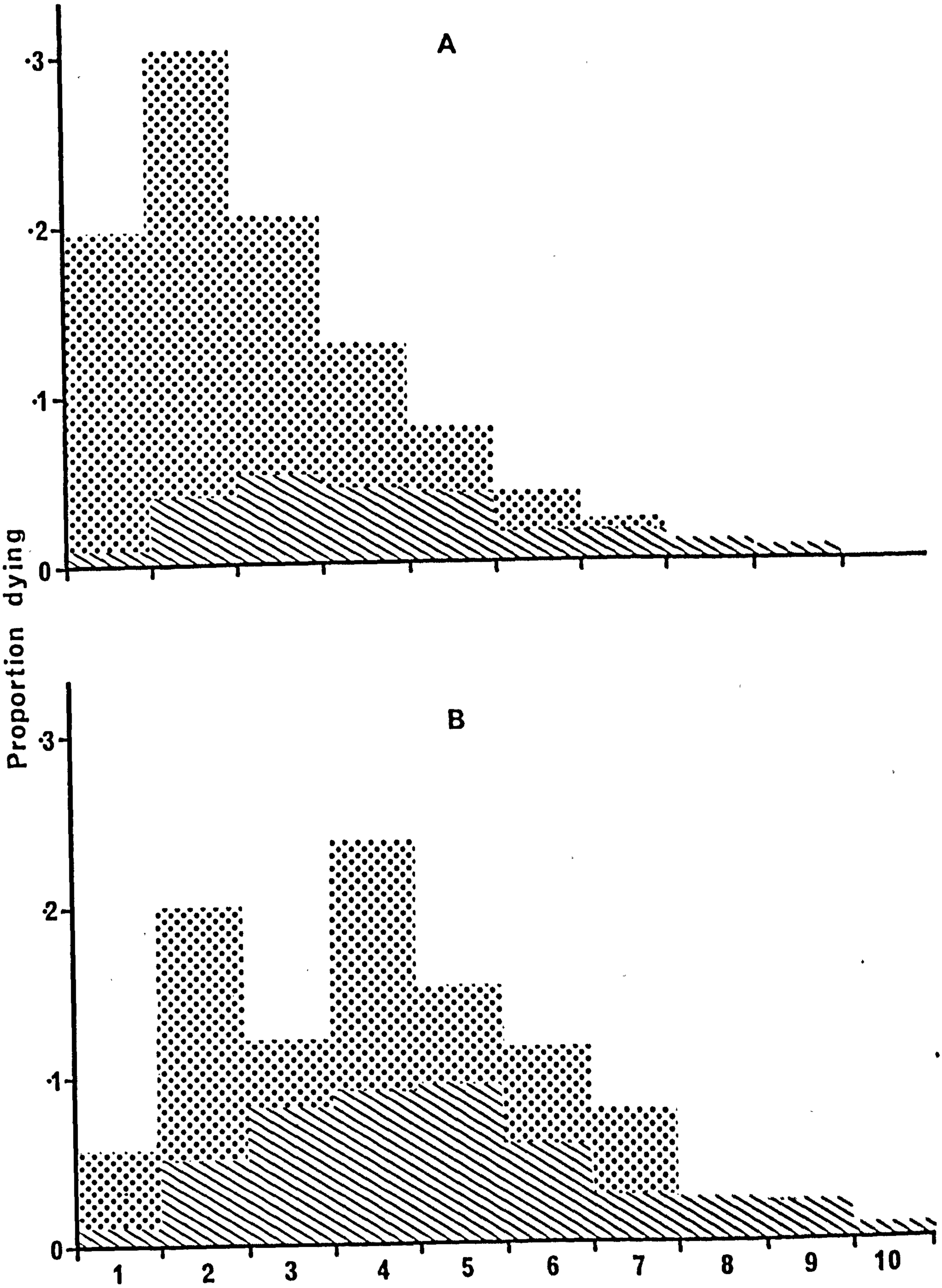
The proportion of adult flukes dying each week.

1. The dots represent the proprtion of mortality attributable to density factors.
2. The diagonal lines represent the proportion of mortality attributable to age dependent factors.
3. The dots plus the diagonal lines represent the total proportion dying each week..

A. Average initial parasite denstiy 145.8 flukes per host.

B. Average initial parasite density 72.4 flukes per host.

(see next page for a description of the method of estimation used)


A
B
Proportion dying
3
2
1
0
1
2
3
4
5
6
7
8
9
10
Weeks

b) Fecundity

In attempting to examine the effect of parasite density on fecundity, the problem of distinguishing density dependent, and age dependent effects, again arises. By looking at the fecundity of parasites over a range of initial densities it was hoped that changes in the normal age dependent pattern of egg production (chapter 3) attributable to density dependent factors could be distinguished.

Fig. 33 and table 11 a-f shows the rate of egg production per hour per surviving fluke at the six densities examined. From the graphs it is clear that there are no major differences in egg production per surviving flukes at densities of between 1 and 72.4 flukes per host bearing in mind the fairly wide confidence limits. In fig. 33a the data is grouped from larger intervals due to the paucity of data. For fig. 33f the data from the 145.8 density class has been combined with that from the 132.4 flukes per host reinfection experiment, in order to increase the quantity of data available. This was possible because it is clear from chapter 6 that there is no observable difference between egg production data from the two classes.

At an initial density of 30 flukes per host and at 72.4 flukes per host the peak of egg production occurs about a week later than at lower initial parasite densities. Also at an initial density of 30 flukes per host, there is a longer "tail" in egg production than at any other density. This was due to a small number of flukes surviving on two hosts which continued to produce eggs at a low rate for longer than at any other density (fig. 33C).

It is apparent from fig. 33F that there is a very considerable difference between the rate of egg production at an average initial parasite density of 139.7 flukes per host and lower initial densities. Hence, there is evidence that the rate of egg prod-

uction per fluke exhibits density dependence at high initial parasite densities. Until the end of the first week post infection egg production per surviving fluke remains well within the range shown at the lower densities. However, instead of the continuing rapid increase in egg production for at least another week, shown by the lower densities at 23°C, the rate of egg production is almost stationary. After three weeks post infection the rate observed is under half the rate shown at lower densities.

The rate recovers however, and after approximately 32 days post infection, it has risen to a level comparable to the lower densities. Subsequently the rate declines in a manner indentical to that displayed at the other densities. These results are shown in comparative form in fig. 34.

The empirical polynomial model described in chapter 4 (equation 14) gives an excellent fit to all the egg production curves ($P < .01$) except at 139.7 flukes initial density per host where $P < .05$ (fig. 35, table 17).

The mean rate of egg production per surviving fluke at the midpoints of successive weeks at each density was determined from the graphs in fig. 33 (table 18). As in chapter 4 the product of this mid-week rate and the proportion of flukes surviving to the midpoints of successive weeks (table 23) gives the egg production per average fluke in successive weeks (fig. 36, table 19).

There are some variations within particular weeks between the one, two and fourteen flukes per host levels. However, when these results are expressed in a cumulative manner (fig. 37, table 20) it is clear that these fluctuations even out to give similar totals. The graph of total egg output per average fluke during the course of infection confirms this point (fig. 39, table 21A).

At 30 flukes per host, egg production per average fluke

reaches a lower peak, and this occurs in the fourth, rather than in the third, week post infection (fig. 36D). This is the result of an interaction between two factors neither of which appeared significant in themselves. A slightly smaller proportion of flukes survived in weeks 3 to 5 (table 38) and the slightly later peak in egg production (fig.33D) compared to lower initial parasite densities.

The cumulative weekly total of eggs per average surviving fluke is consistantly lower except in weeks one and two (fig. 37). Total cumulative egg production per average fluke falls from 59-65 eggs at lower initial densities to 53 at 30 flukes (fig.39, table 21).

Egg production per average surviving fluke in the 72.4 initial density class shows a much more substantial fall. This is mainly due to the decreased survival of the flukes, but again, due partly to the rather later peak in egg production per surviving fluke (figs. 36E, 37E).

In the 139.7 initial density class, both the much reduced survival of the flukes, and substantial reduction in egg production per surviving fluke, result in an extremely large drop in egg production per surviving fluke (figs. 36F, 37F).

Fig. 39 and table 21A show the relationship between total cumulative egg production per average fluke (λ) during the course of infection and initial parasite density. This shows a steady decline with increasing density above 14 flukes per host.

Using equation 16 the average egg production per host during the course of infection for each parasite density was determined. Fig. 38 (table 21b) show that maximum egg output per host is obtained at about the 72.4 initial parasite density. Above this level the effects of the declining rate of egg production per surviving fluke, and the decline in parasite survival outweigh the increase in initial parasite numbers.

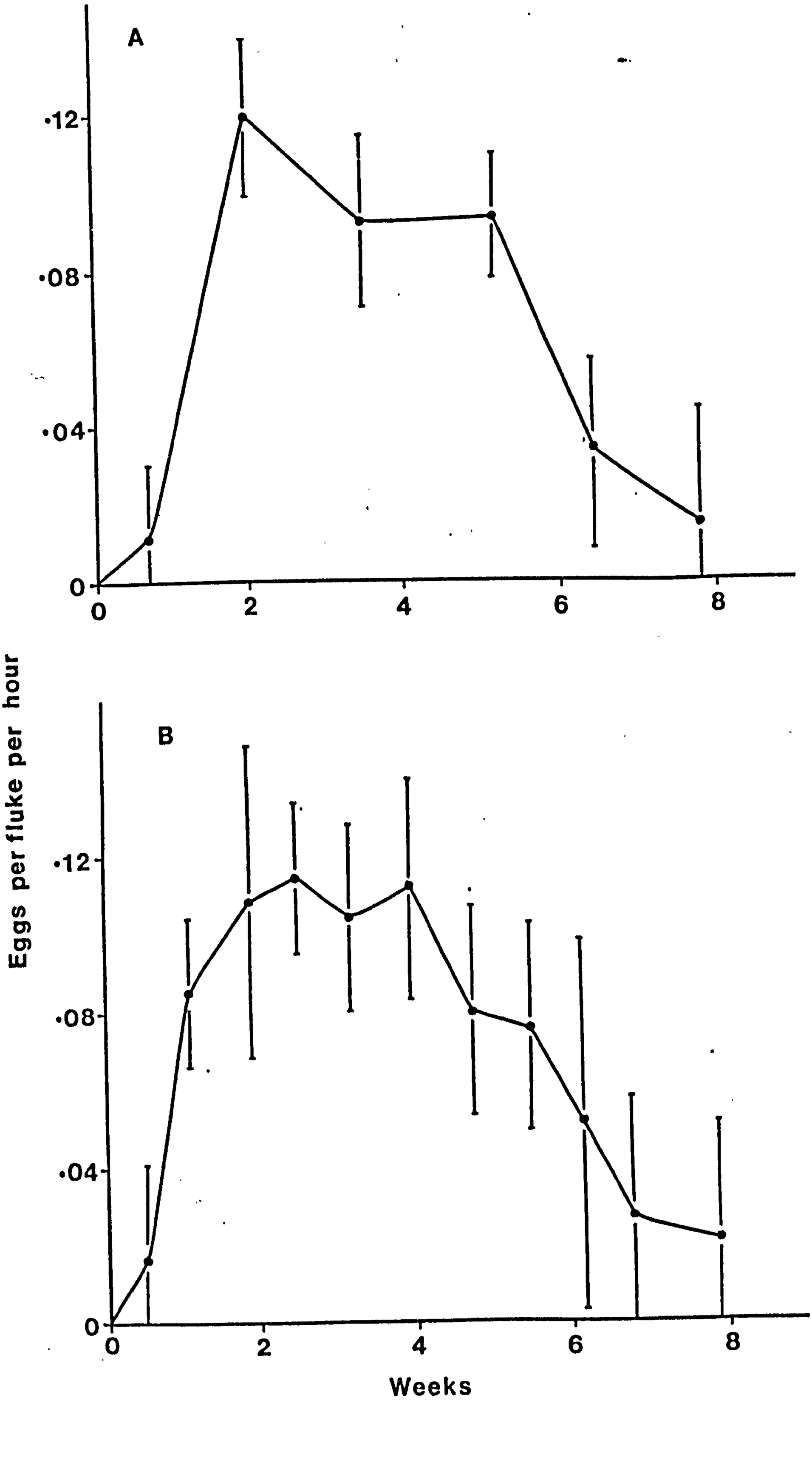
FIG.33.

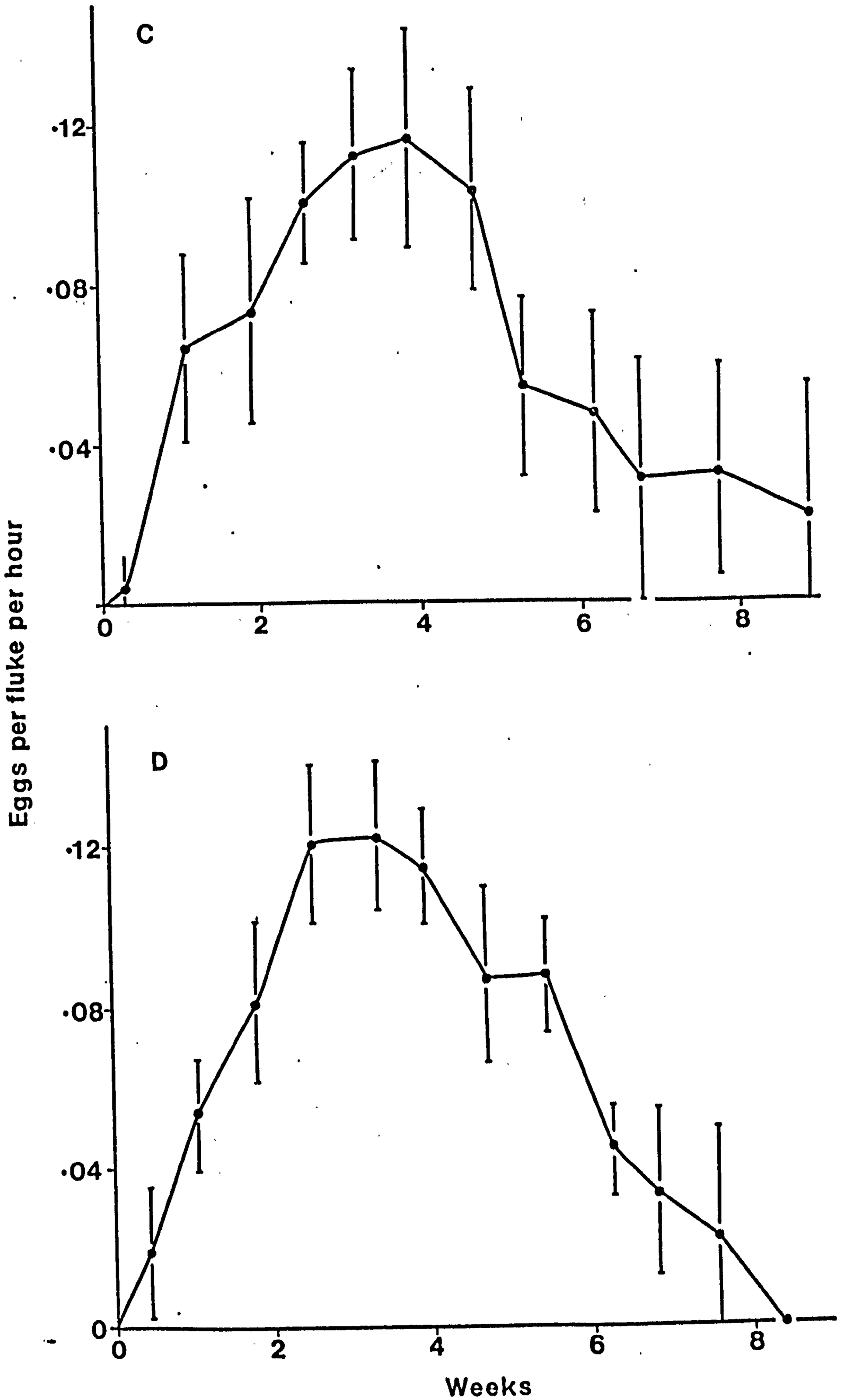
Fig. 33

Egg production per surviving fluke per hour against time at 23°C.

1. The vertical bars show the 95% confidence limits round the observed points.

	Initial parasite densities
A.	1
B.	2
C.	14
D.	30
E.	72.4 (average)
F.	145.8 (average)


A
·12
·08
·04
0
0
2
4
6
8
Eggs per fluke per hour
B
·12
·08
·04
0
0
2
4
6
8
Weeks


C
·12
·08
·04
Eggs per fluke per hour
0
2
4
6
8
D
·12
·08
·04
0
0
2
4
6
8
Weeks

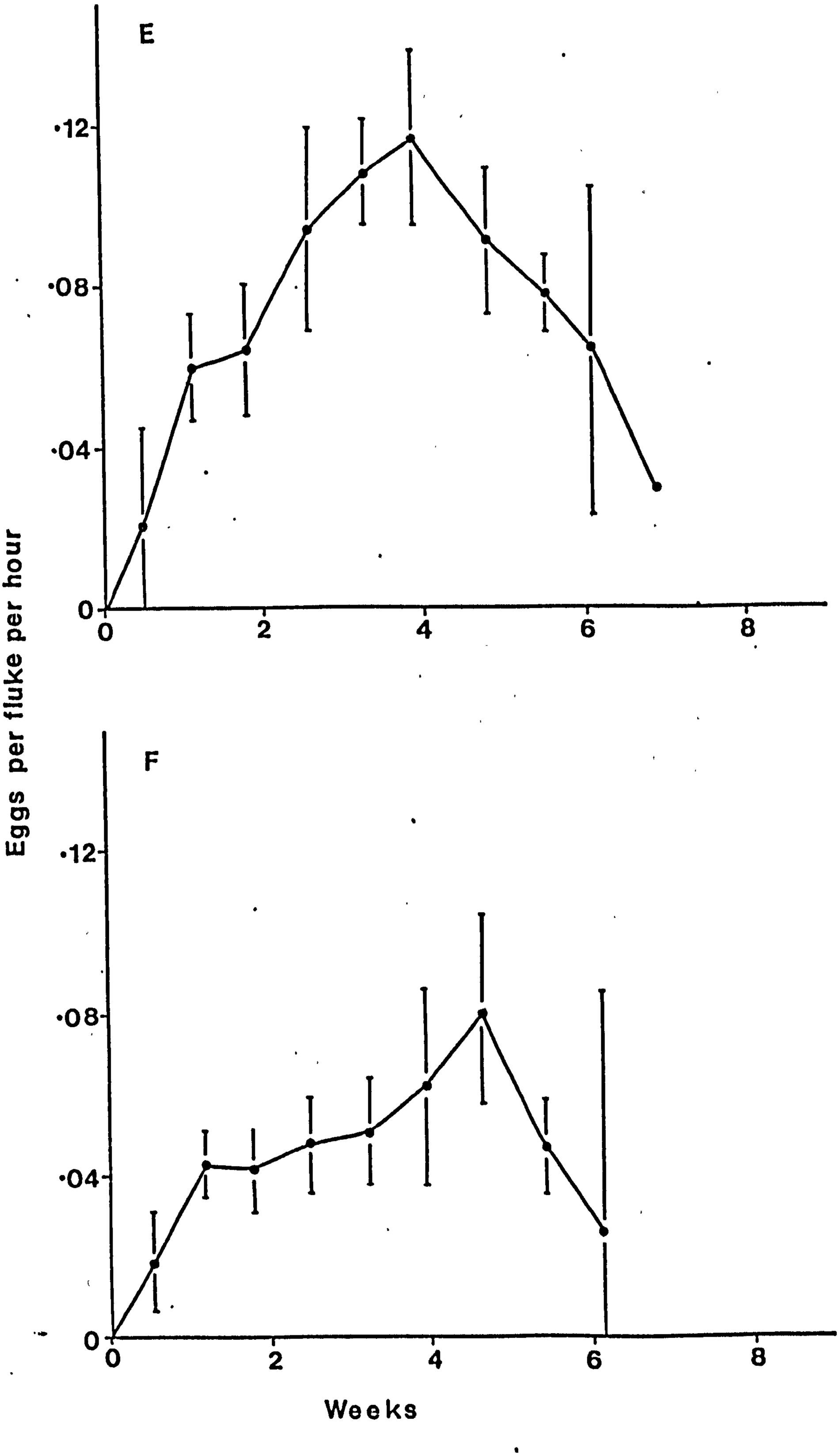

E
·12
·08
·04
0
0
2
4
6
8
Eggs per fluke per hour
F
·12
·08
·04
0
0
2
4
6
8
Weeks

FIG.34.

Fig. 34

Egg production per surviving parasite per hour, at the mid-points of successive weeks post infection for five initial parasite densities per host.

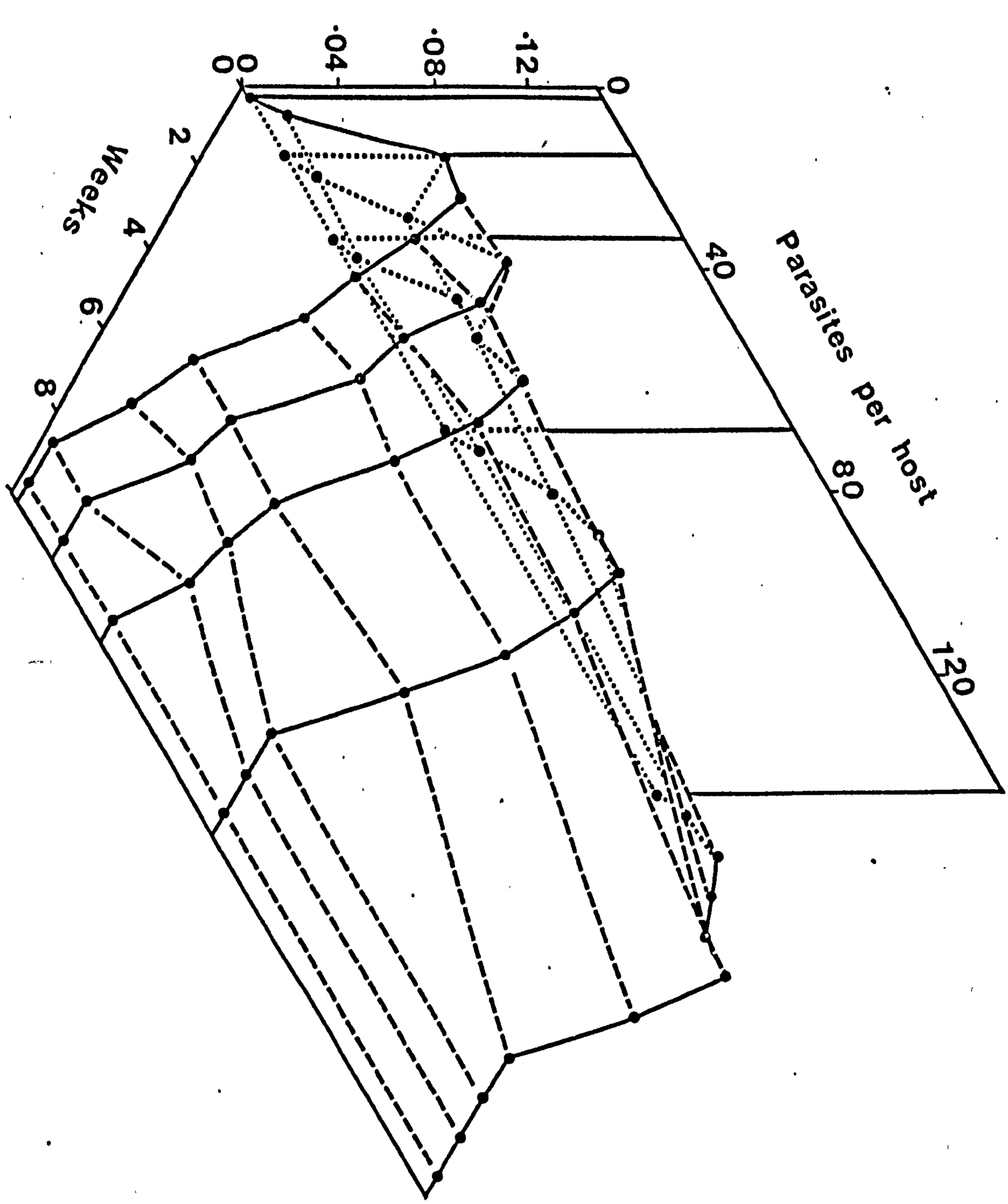

Eggs per fluke per hour
0
·04
·08
·12
0
Weeks
2
4
6
8
40
Parasites per host
80
120

FIG.35.

Fig. 35

Egg production per surviving fluke per hour against time.

1. The solid circles represent the observed rate of egg production at a series of consecutive points in time.
2. The solid line is the rate predicted by an empirical second order polynomial model (equation 14).

	Initial parasite densities
A.	1
B.	2
C.	14
D.	30
E.	72.4 (average)
F.	145.8 (average)

The values of the polynomial coefficients and the fit of the polynomial curves to the observed data see table 17.

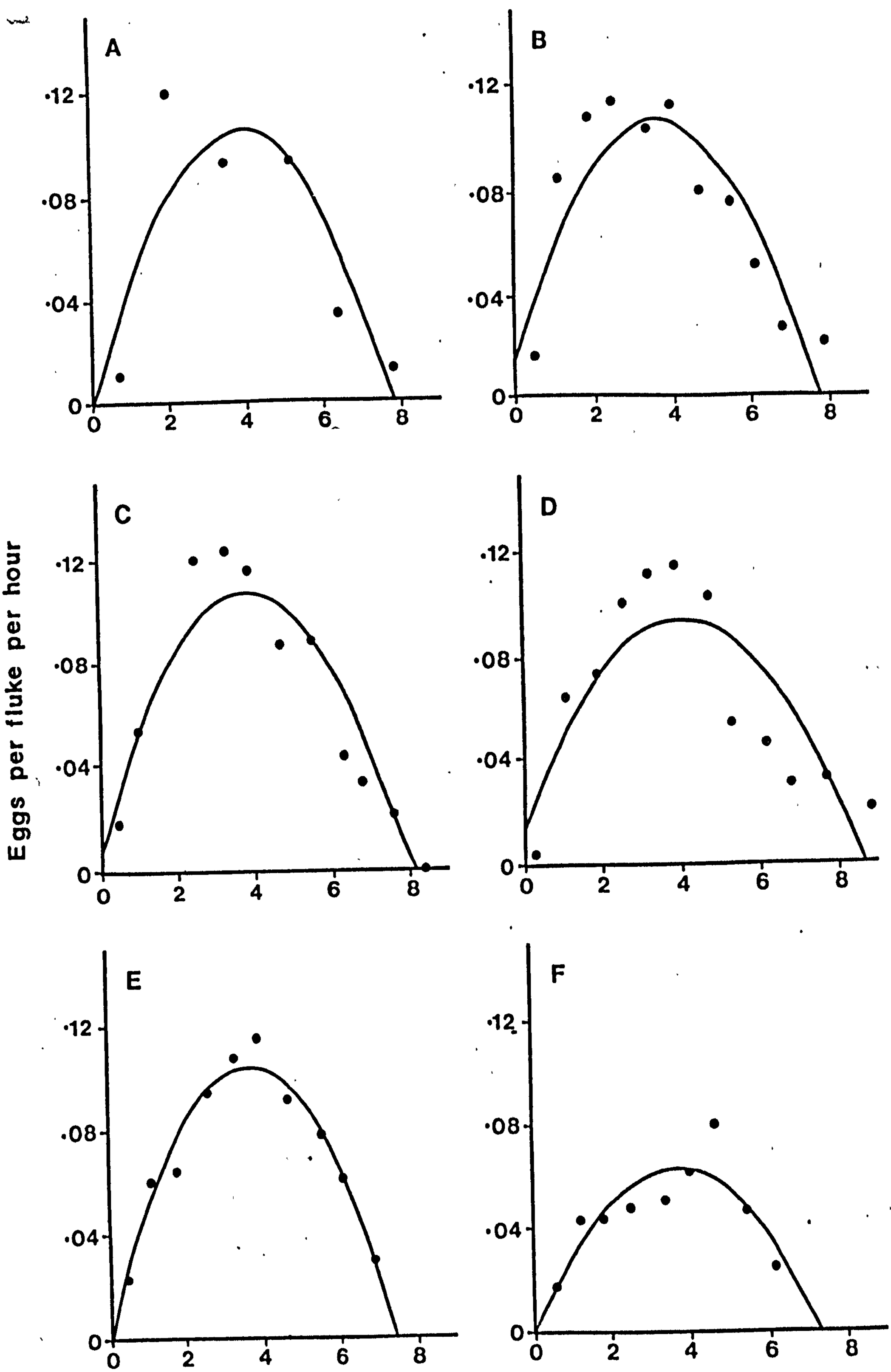

A
B
C
D
E
F
·12
·08
·04
0
0
2
4
6
8
Eggs per fluke per hour
Weeks

FIG.36.

Fig. 36

Egg production per average fluke against time over a range of initial parasite densities.

	Initial parasite density (per host)
A.	1
B.	2
C.	14
D.	30
E.	72.4 (average)
F.	145.8 (average)

Egg production per average fluke is the product of the mean egg production per surviving fluke and the proportion of flukes surviving at the midpoints of successive weeks.

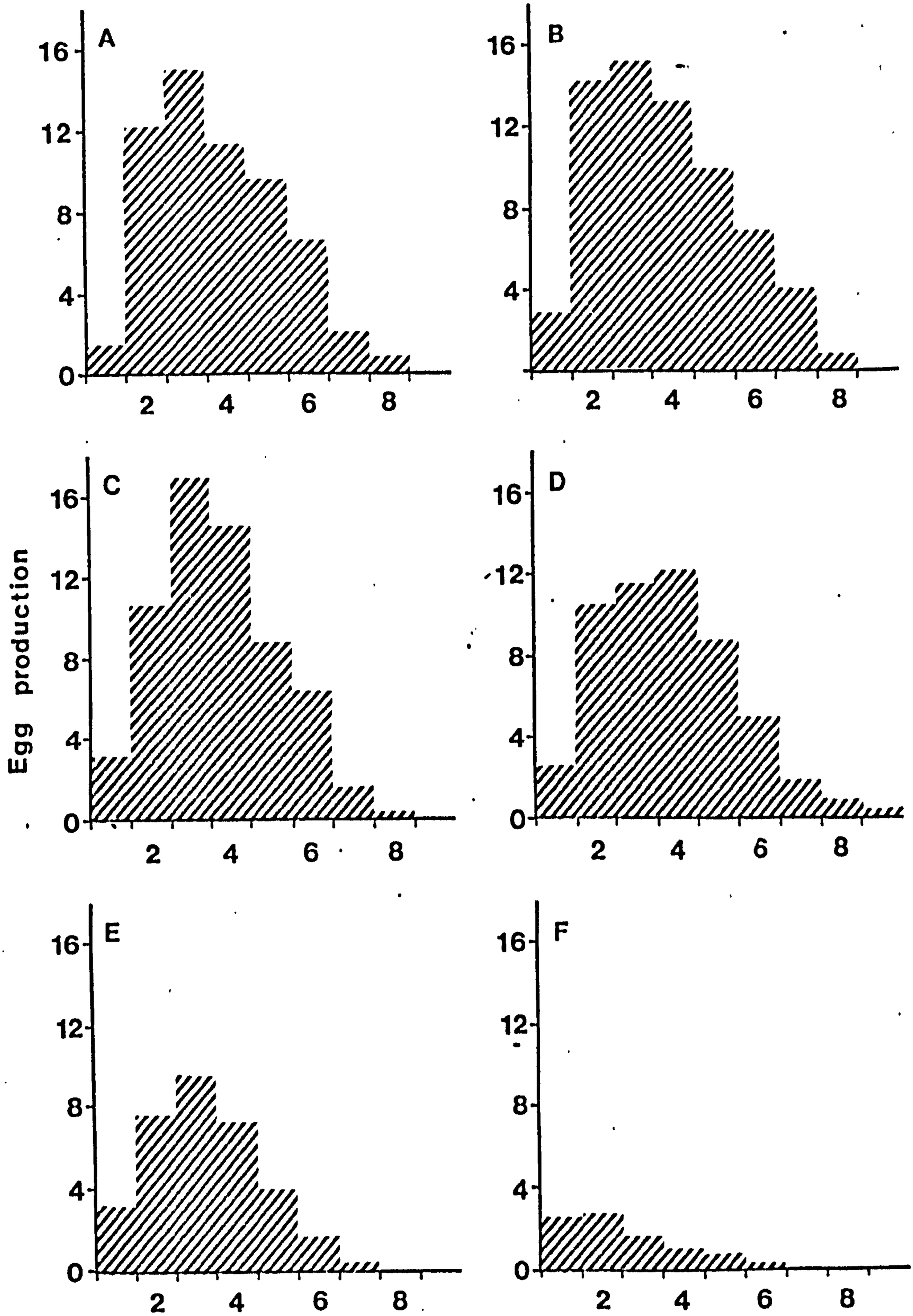

A
B
C
D
E
F
16
12
8
4
0
2
4
6
8
Egg production
Weeks

FIG.37.

Fig. 37

Cumulative egg production per average fluke against time.

Initial parasite density
(per host)

A. 1

B. 2

C. 14

D. 30

E. 72.4 (average)

F. 145.8 (average)

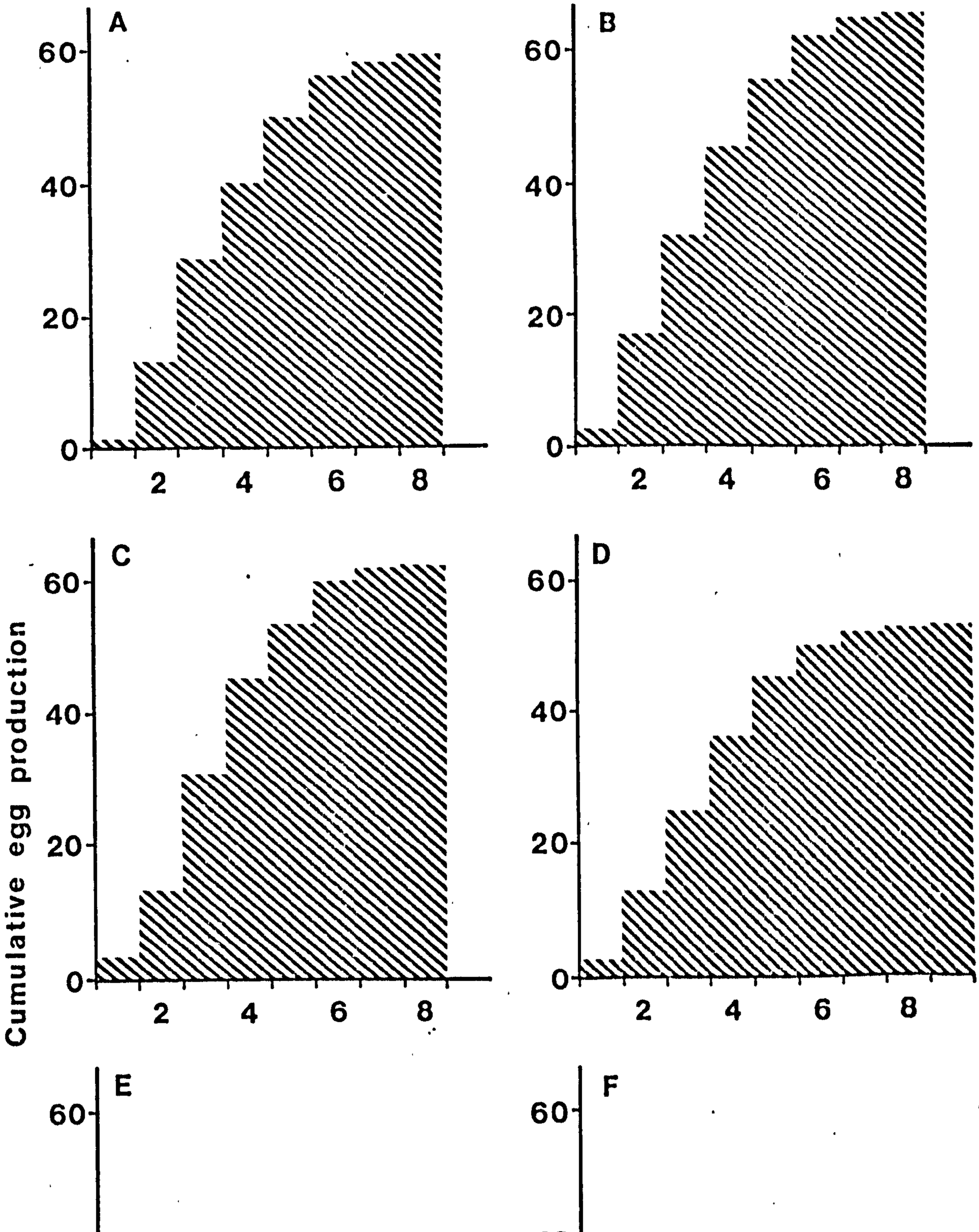

A
B
C
D
E
F
60
40
20
0
2
4
6
8
Cumulative egg production
Weeks

FIG.38.

Fig. 38.

Total cumulative mean egg production by all the parasites per host during the course of infection against initial parasite density.

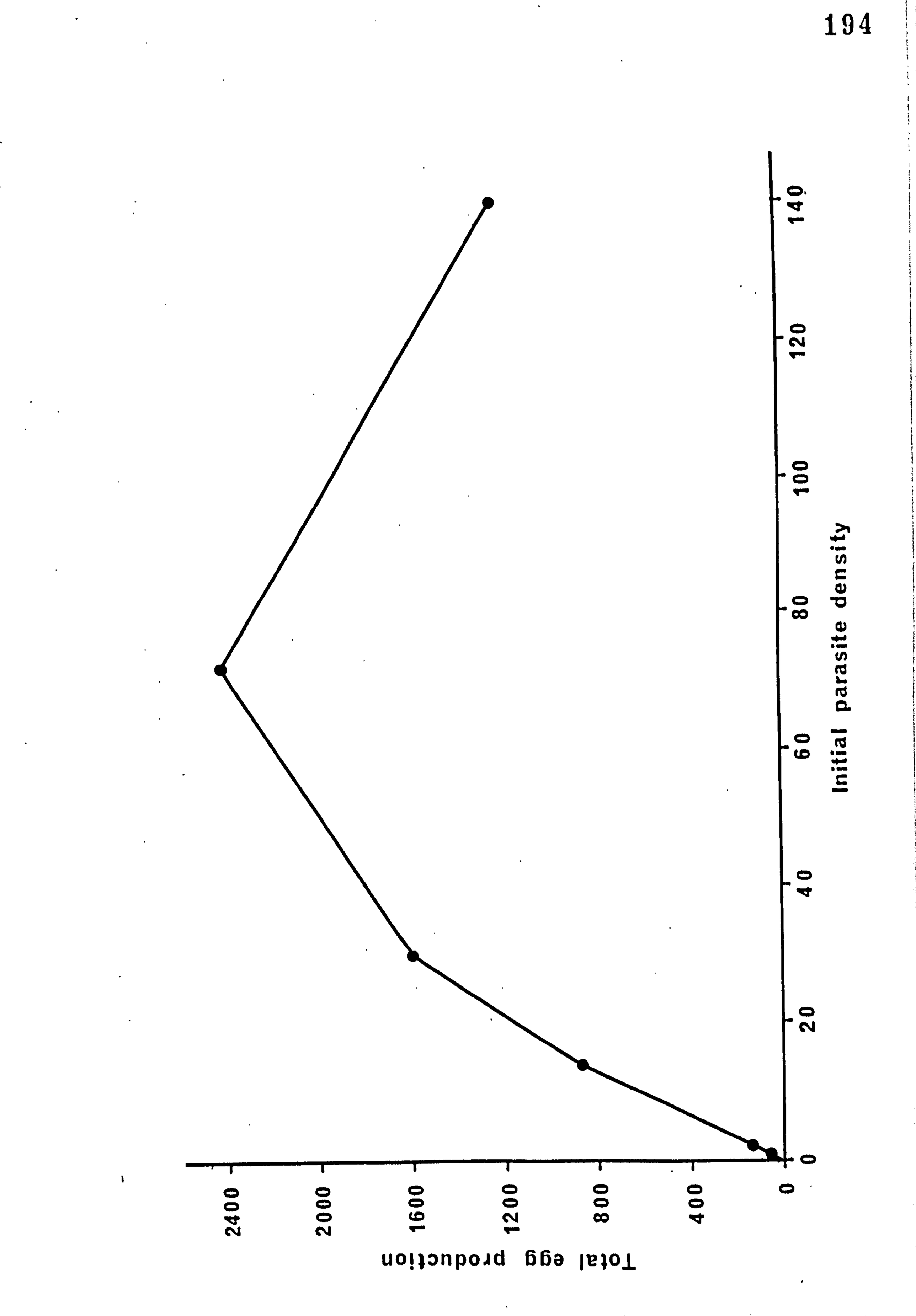

Total egg production
2400
2000
1600
1200
800
400
0
Initial parasite density
0
20
40
60
80
100
120
140

FIG.39.

Fig. 39

Total cumulative egg production per average fluke against initial parasite density per host.

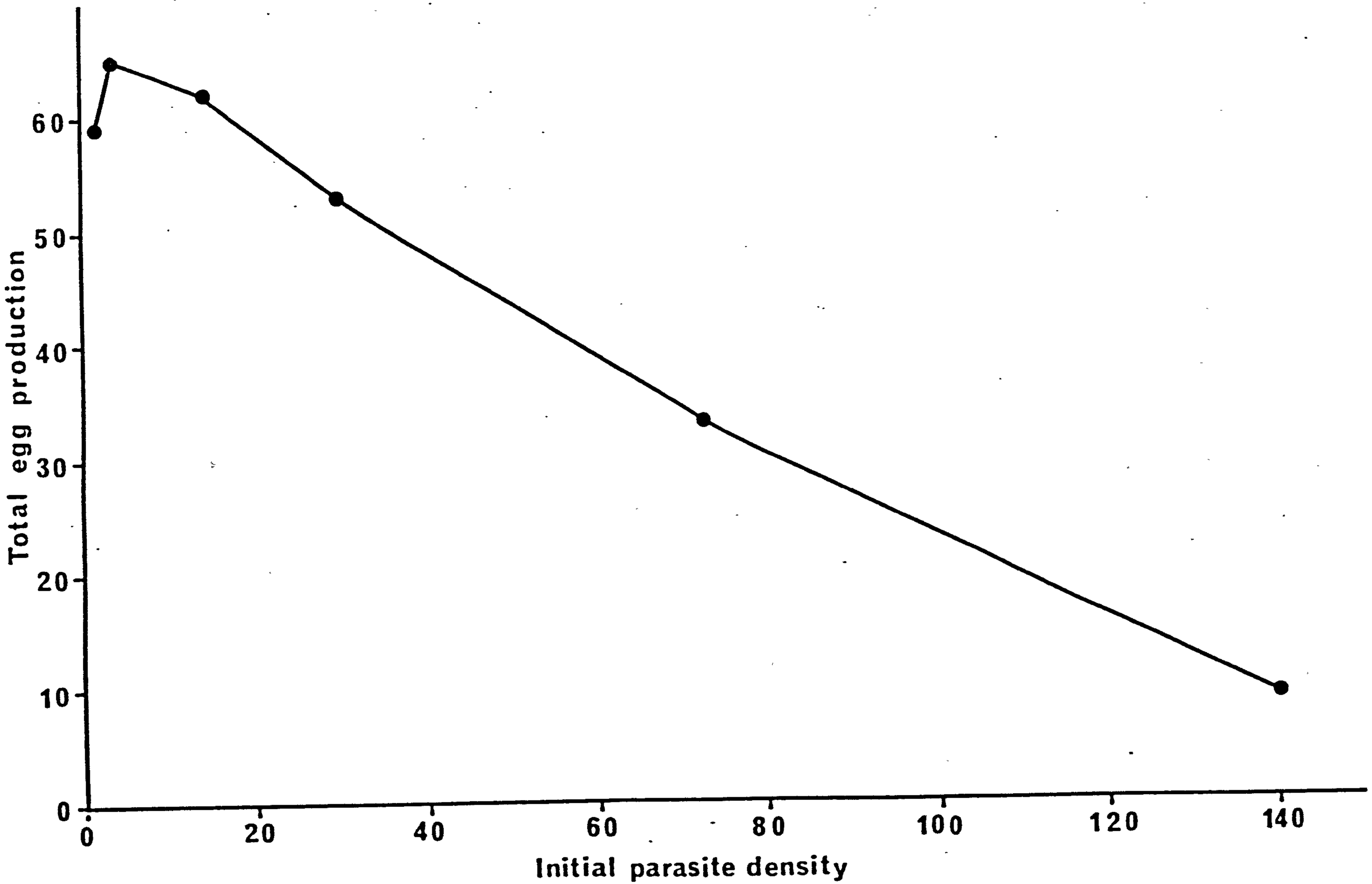

Total egg production
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140
Initial parasite density

Table 14 The values of coefficients a and b and correlation coefficients for the empirical model (equation 2) fitted to the observed instantaneous death rates of parasites at six different initial densities of parasites per host.

Initial parasite densities	1	2	14	30	72.4 (average)	145.8 (average)
Coefficient a (intercept)	.03569	.3228	.02803	.04941	.1352	.4287
Coefficient b (slope)	.3646	.3780	.5129	.3543	.2934	.1044
Correlation Coefficient r	.9504	.9152	.9822	.9591	.9402	.5717
Degrees of freedom	9	9	8	9	8	7
Level of significance	$<.001$	$<.001$	$<.001$	$<.001$	$<.001$	$>.1$

Table 15 Proportion of parasites on individual heavily infected hosts (28-32 mm size class) surviving to the midpoints of successive weeks at 23°C.

Host number	1	2	3	4	5	6	9R	8R	7R	7	8	10R	9	11R	10	11
Initial number of parasites	56	60	80	82	84	118	120	122	127	129	135	140	147	153	169	177
Time (weeks)																
0.5	.98	.98	.99	.96	.81	.89	.83	.89	.67	.87	.88	.83	.77	.64	.81	.97
1.5	.79	.89	.84	.87	.56	.52	.53	.46	.24	.24	.32	.47	.52	.11	.07	.63
2.5	.67	.76	.73	.80	.18	.38	.35	.26	.11	.05	.12	.32	.38	.05	.04	.22
3.5	.48	.58	.33	.54	.02	.15	.24	.15	.02	.02	.04	.21	.35	.02	.02	.05
4.5	.29	.45	.17	.29	.00	.02	.06	.04	.001	.02	.001	.14	.25	.01	.00	.01
5.5	.11	.29	.04	.20	.00	.003	.05	.01	.00	.02	.00	.06	.18	.003	.00	.01
6.5	.01	.12	.03	.10	.00	.00	.03	.002	.00	.01	.00	.03	.11	.00	.00	.00
7.5	.00	.06	.01	.06	.00	.00	.01	.00	.00	.03	.00	.02	.04	.00	.00	.00
8.5	.00	.01	.00	.03	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.00	.00
9.5	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00

R denotes reinfected fish.

Table 16 Instantaneous death rates of parasites on individual heavily infected hosts (28-32 mm size class) at the midpoints of successive weeks at 23°C.

Host number	1	2	3	4	5	6	9R	8R	7R	7	8	10R	9	11R	10	11
Initial number of parasites	56	60	80	82	84	118	120	122	127	129	135	140	147	153	169	177
Time (weeks)																
.25	.040	.040	.030	.082	.421	.233	.397	.233	.801	.278	.256	.373	.523	.893	.422	.060
1	.216	.096	.159	.098	.369	.537	.449	.660	1.027	1.288	1.012	.569	.393	1.761	2.449	.432
2	.165	.158	.140	.084	1.135	.312	.415	.570	.780	1.569	.981	.384	.314	.788	.560	1.052
3	.333	.270	.794	.393	2.179	.930	.377	.550	1.705	.916	1.099	.421	.082	.916	.693	1.482
4	.504	.254	.663	.622		2.015	1.386	.511	2.996	.000	3.689	.405	.336	.693		1.609
5	.969	.439	1.447	.372		1.897	.405	1.861		.000		.847	.329	1.609		
6	2.398	.883	.288	.693			.654	1.946		.693		.799	.492			
7		.693	1.499	.511			1.649			1.204		.739	1.012			
8		1.792		.693									1.386			
9				2.300												
Coefficient a	.0599	.0547	.0722	.0800	.2765	.2238	.3416	.2820	.6382	.2236	.3186	.3681	.7077	1.0223	.8411	.1122
Coefficient b	.5974	.4230	.4460	.3311	.6710	.4594	.1597	.3033	.3349	.1564	.5609	.1071	.0638	.0068	.0354	.8000

Table 17 The values of coefficients α, β and γ and significance of fit of a second order polynomial model (equation 14) to observed data for the rate of egg production per surviving fluke against time at six different initial densities of parasite per host.

Initial parasite densities	1	2	14	30	72.4 (average)	145.8 (average)
Coefficient α (intercept)	-.00046	.03105	.01504	.02289	-.00501	.00072
Coefficient β	.05348	.04103	.04717	.03439	.05894	.03369
Coefficient γ	-.00686	-.00577	-.00615	-.00426	-.00785	-.00461
F value	3.618	11.807	26.123	8.073	70.806	6.695
Degrees of freedom	2, 3	2, 8	2, 9	2, 9	2, 7	2, 8
Level of significance	Not significant	$<.01$	$<.01$	$<.01$	$<.01$	$<.05$

Table 18 The rate of egg production per surviving fluke per hour at the midpoints of successive weeks estimated from fig. 33.

Initial parasite densities	1	2	14	30	72.4	139.7
Weekly midpoints						
0.5	.009	.017	.019	.015	.020	.018
1.5	.080	.097	.067	.068	.061	.042
2.5	.111	.114	.120	.086	.090	.048
3.5	.094	.107	.120	.114	.110	.055
4.5	.094	.090	.095	.107	.099	.076
5.5	.079	.076	.087	.080	.078	.045
6.5	.033	.040	.040	.039	.046	.000
7.5	.019	.024	.034	.032	.000	
8.5	.000	.000	.000	.024		
9.5				.000		

Table 19 Mean egg production per surviving fluke per week times the proportion of flukes surviving at the midpoints of each week (table 18).

Initial parasite densities	1	2	14	30	72.4	139.7
Weeks						
1	1.50	2.84	3.16	2.49	3.17	2.49
2	12.15	14.18	10.58	10.46	7.62	2.64
3	15.00	15.16	16.91	11.53	9.48	1.65
4	11.34	13.10	14.45	12.16	7.22	1.08
5	9.68	9.91	8.73	8.72	3.98	0.68
6	6.64	6.89	6.39	4.89	1.67	0.24
7	2.00	2.44	1.63	1.70	0.40	
8	0.79	0.75	0.37	0.81		
9				0.31		

Table 20 Cumulative mean egg production per surviving fluke per week times the proportion of flukes surviving at the midpoint of each week.

Initial parasite densities	1	2	14	30	72.4	139.7
Weeks						
1	1.5	2.84	3.16	2.49	3.17	2.49
2	13.65	17.02	13.74	12.95	10.79	5.13
3	28.65	32.18	30.65	24.48	20.27	6.78
4	39.99	45.28	45.10	36.64	27.49	7.86
5	49.67	55.19	53.83	45.36	31.47	8.54
6	56.31	62.08	60.22	50.25	33.14	8.78
7	58.31	64.52	61.85	51.95	33.54	
8	59.10	65.27	62.22	52.76		
9				53.07		

Table 21

	A	B
Initial parasite densities	Total cumulative egg production per surviving fluke per week times the proportion of flukes surviving at the midpoint of week (equals egg production per average fluke).	Total cumulative mean egg production by all the parasites per host during the course of infection.
1	59.10	59.10
2	65.27	130.50
14	62.22	871.1
30	53.07	1591.1
72.4	33.54	2428.3
139.7	8.78	1226.6

Table 22 The proportions of mortality attributable to age and to density at an initial parasite density of 145.8 per host.

	a	b	c	d (b x c)	m_1 (d - c)	M (c + 1 - c)	m_2 (M - m_1)
Time (weeks)	Proportion of parasites surviving at an initial density of 14 per host.	Chance of parasites surviving to the midpoint of the next week (%)	Proportion of parasites surviving at an initial density of 145.8 per host.	Predicted proportion of parasites surviving at an initial density of 145.8 per host.	Change in proportion of flukes attributable to density.	Total change in proportion of flukes surviving.	Change in proportion of flukes attributable to age
0	1.000	99.0	1.000				
0.5	.990	94.9	.803	.990	-.187	.197	.010
1.5	.940	89.3	.499	.762	-.263	.304	.041
2.5	.839	85.5	.294	.446	-.152	.205	.053
3.5	.717	76.3	.163	.251	-.088	.131	.043
4.5	.547	79.9	.085	.124	-.039	.078	.039
5.5	.437	55.6	.041	.068	-.027	.044	.017
6.5	.243	26.7	.019	.023	-.004	.022	.016
7.5	.065	20.0	.008	.005	+.002	.011	.009
8.5	.013	7.7	.003	.002	+.001	.005	.004
9.5	.001	0.0	.000	.000	..000	.000	.000

Table 23 The mean proportion of parasites surviving at a series of consecutive points in time. Observed values and values calculated from an empirical model (equation 4).

Initial parasite density	1		2		14		30		72.4		139.7 *		132.4		145.8	
Weekly midpoints	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
0.5	.991	.981	.993	.982	.990	.984	.988	.973	.943	.930	.865	.803	.774	.764	.865	.802
1.5	.904	.931	.870	.937	.940	.939	.916	.907	.744	.775	.386	.499	.363	.432	.386	.498
2.5	.804	.864	.791	.874	.839	.867	.798	.820	.627	.607	.196	.294	.216	.234	.196	.294
3.5	.718	.777	.729	.790	.717	.760	.635	.710	.391	.438	.107	.163	.129	.121	.107	.163
4.5	.613	.666	.656	.682	.547	.610	.485	.579	.240	.282	.051	.085	.0569	.059	.051	.085
5.5	.500	.533	.540	.550	.437	.422	.364	.432	.127	.157	.036	.041	.0266	.027	.035	.041
6.5	.361	.387	.362	.402	.243	.228	.259	.285	.051	.071	.020	.019	.0111	.012	.020	.019
7.5	.248	.244	.187	.254	.065	.082	.150	.156	.029	.025	.007	.008	.0046	.005	.007	.008
8.5	.132	.126	.119	.130	.013	.015	.076	.068	.008	.006	.002	.003			.002	.003
9.5	.070	.048	.067	.049	.001	.001	.029	.020	.001	.001						
10.5	.009	.012	.007	.012			.005	.004								

* mean of 132.4 and 145.8 classes.

Table 24 The proportion of mortality attributable to age and to density at an initial parasite density of 72.4 flukes per host (average).

	a	b	c	d (b x c)	m_1 (d - c)	M (c + 1 - c)	m_2 (M - m_1)
Time (weeks)	Proportion of parasites surviving at an initial density of 14 per host.	Chance of parasites surviving to the midpoint of the next week (%).	Proportion of parasites surviving at an initial density of 72.4 per host.	Predicted proportion of parasites surviving at an initial density of 72.4 per host.	Change in proportion of flukes attributable to density.	Total change in proportion of flukes surviving.	
0	1.000	99.0	1.000				
0.5	.990	94.9	.943	.990	-.047	.057	.010
1.5	.940	89.3	.744	.895	-.151	.199	.048
2.5	.839	85.5	.627	.664	-.037	.117	.080
3.5	.717	76.3	.391	.536	-.145	.236	.091
4.5	.547	79.9	.240	.248	-.058	.151	.093
5.5	.437	55.6	.127	.183	-.056	.113	.057
6.5	.243	26.7	.051	.101	-.050	.076	.026
7.5	.065	20.0	.029	.029	.000	.022	.022
8.5	.013	7.7	.008	.006	+.002	.021	.019
9.5	.001	0.0	.001	.001	.000	.007	.007

Table 25A Egg output per fluke per hour at an initial density of 1 fluke per host.

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-10	0.71	.0111	.0183	6	.0192
11-20	2.00	.1197	.0188	6	.0197
21-30	3.50	.0931	.0368	13	.0222
31-40	5.17	.0944	.0227	10	.01624
41-50	6.43	.0337	.0242	6	.0247
51-60	7.83	.0144	.0279	6	.0297

Table 25B Egg output per fluke per hour at an initial density of 2 flukes per host.

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits.
1-5	.50	.0160	.0225	6	.0236
6-10	1.09	.0852	.0319	13	.0193
11-15	1.86	.1079	.0441	7	.0406
16-20	2.50	.1136	.0336	14	.0194
21-25	3.36	.1039	.0284	8	.0237
26-30	3.97	.1116	.0391	10	.0279
31-35	4.76	.0804	.0358	9	.0274
36-40	5.49	.0763	.0435	12	.0276
41-45	6.14	.0509	.0507	7	.0469
46-50	6.78	.0270	.0365	8	.0305
51-60	7.87	.0212	.0318	7	.0294

Table 25C Egg output per fluke per hour at an initial density of 14 flukes per host.

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-5	.44	.0189	.0156	6	.0164
6-10	1.04	.0539	.0172	11	.0116
11-15	1.83	.0810	.0278	10	.0199
16-20	2.54	.1198	.0214	7	.0198
21-25	3.34	.1232	.0304	13	.0184
26-30	3.91	.1146	.0228	12	.0145
31-35	4.69	.0865	.0342	12	.0217
36-40	5.46	.0876	.0182	9	.0140
41-45	6.27	.0436	.0167	11	.0112
46-50	6.83	.0330	.0249	8	.0206
51-55	7.59	.0210	.0235	5	.0292
56-60	8.39	.000	-	4	-

Table 25D Egg output per fluke per hour at an initial density of 30 flukes per host.

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-5	.29	.0043	.0051	4	.0081
6-10	1.07	.0643	.0277	8	.0231
11-15	1.89	.0730	.0338	8	.0282
16-20	2.56	.1012	.0226	11	.1152
21-25	3.23	.1124	.0297	10	.0213
26-30	3.91	.1160	.0322	8	.0269
31-35	4.74	.1028	.0345	10	.0247
36-40	5.29	.0542	.0267	8	.0223
41-45	6.19	.0469	.0286	7	.0248
46-50	6.84	.0307	.0326	7	.0301
51-60	7.75	.0330	.0344	9	.0264
61-70	8.87	.0214	.0366	7	.0339

Table 25E Egg output per fluke per hour at an initial density of 72.4 flukes per host.

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-5	.51	.0215	.0274	7	.0254
6-10	1.14	.0600	.0154	8	.0128
11-15	1.81	.0641	.0173	7	.0159
16-20	2.60	.0937	.0332	9	.0256
21-25	3.27	.1079	.0141	7	.0130
26-30	3.90	.1161	.0237	7	.0220
31-35	4.69	.0912	.0246	10	.0176
36-40	5.54	.0776	.0071	5	.0089
41-45	6.10	.0628	.0262	4	.0417
46-50	6.89	.0300	-	1	-

Table 25F Egg output per fluke per hour at an average initial density of 139.7 flukes per host.

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-5	.54	.0184	.0208	13	.0126
6-10	1.20	.0429	.0144	16	.0079
11-15	1.77	.0420	.0221	18	.0110
16-20	2.50	.0484	.0217	16	.0115
21-25	3.27	.0513	.0232	14	.0134
26-30	3.94	.0621	.0368	11	.0247
31-35	4.64	.0795	.0212	6	.0223
36-40	5.44	.0465	.0202	9	.0115
41-45	6.14	.0260	.0393	4	.0625

Table 26 Mean proportion of parasites surviving at a series of consecutive points in time. Observed values and values calculated using coefficients a′ and b′ calculated from the exponential model (equations 19, 20) fitted to coefficients a and b.

Initial parasite density	1		2		14		30		72.4		132.7		145.8	
Weekly midpoints	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
0.5	.991	.984	.993	.984	.979	.990	.986	.972	.943	.937	.774	.809	.865	.759
1.5	.904	.939	.870	.938	.925	.940	.916	.903	.744	.804	.363	.509	.386	.418
2.5	.804	.872	.791	.870	.849	.839	.798	.814	.627	.665	.216	.304	.196	.217
3.5	.718	.776	.729	.774	.749	.717	.635	.704	.391	.525	.129	.170	.107	.105
4.5	.613	.646	.656	.645	.617	.547	.485	.575	.240	.383	.057	.088	.051	.047
5.5	.500	.484	.540	.484	.469	.437	.364	.434	.127	.274	.027	.042	.036	.020
6.5	.361	.307	.362	.308	.309	.243	.259	.292	.051	.175	.011	.019	.020	.007
7.5	.248	.150	.187	.152	.167	.065	.150	.168	.029	.100	.007	.007	.007	.003
8.5	.132	.048	.119	.050	.066	.013	.076	.078	.008	.050			.002	.001
9.5	.070	.008	.067	.009	.017	.001	.029	.026	.001	.021				
10.5	.009	.001	.007	.001			.005	.006						

Table 27 Instantaneous death rates at a series of consecutive points in time.

obs. - rates calculated from observed survival data.

calc. - calculated from exponential model (equation 2) using coefficient a and b.

calc.′ - calculated from coefficients a ′and b′ derived by fitting an exponential model (equation 19, 20- to a and b.

Initial parasite densities	1			2			14			30		
Time (weeks)	obs.	calc.	calc.′	obs.	calc.	calc.′	obs.	calc.	calc.′	obs.	calc.	calc.′
0.25	.0173	.0428	.0329	.0143	.0355	.0336	.0210	.0318	.0421	.0242	.0452	.0572
1	.0920	.0514	.0466	.1321	.0471	.0473	.0518	.0472	.0568	.0757	.0704	.0734
2	.1172	.0740	.0738	.0947	.0687	.0745	.1137	.0781	.0847	.1379	.1004	.1025
3	.1135	.1066	.1170	.0827	.1003	.1175	.1572	.1304	.1261	.2285	.1431	.1431
4	.1581	.1534	.1854	.1054	.1464	.1853	.2707	.2178	.1878	.2695	.2038	.1997
5	.2038	.2209	.2938	.1941	.2137	.2922	.2245	.3638	.2798	.2870	.2906	.2787
6	.3257	.3181	.4660	.3974	.3118	.4608	.5869	.6077	.4168	.4862	.4141	.3890
7	.3754	.4581	.738	.6625	.4551	.7265	1.3187	1.0149	.6208	.5462	.5902	.5429
8	.6306	.6596	1.169	.4567	.6641	1.1456	1.6092	1.6949	.9248	.6799	.8411	.7577
9	.6343	.9498	1.853	.5687	.9692	1.8060	2.5699	2.8308	1.3775	.9634	1.1988	1.0575
10	2.0513	1.3677	2.937	2.246	1.4144	2.843				1.7578	1.7086	1.4760
Intercept		.03567	.02938		.03228	.02998		.02803	.03815		.04941	.05262
Slope		.3646	.4605		.3780	.4554		.51291	.3985		.3543	.3334

Table 27 Instantaneous death rates at a series of consecutive points in time.

Cont. obs. - rates calculated from observed survival data.
calc. - calculated from exponential model (equation 2) using coefficients a and b.
calc′. - calculated from coefficients a′ and b′ derived by fitting an exponential model (equations 19, 20) to a and b.

Initial parasite densities	72.4			132.7			145.8		
Time (weeks)	obs.	calc.	calc′.	obs.	calc.	calc′.	obs.	calc.	calc′.
0.25	.1176	.1455	.1299	.5124	.5389	.4228	.2901	.4400	.5513
1	.2373	.1813	.1518	.7572	.5697	.4580	.8069	.4759	.5901
2	.1709	.2431	.1870	.5191	.6135	.5095	.6767	.5282	.6462
3	.4371	.3260	.2302	.5155	.6607	.5669	.6110	.5864	.7076
4	.4895	.4372	.2834	.8185	.7115	.6306	.7363	.6509	.7749
5	.6308	.5863	.3489	.7604	.7662	.7016	.3623	.7225	.8485
6	.9077	.7862	.4296	.8740	.8252	.7805	.5639	.8020	.9292
7	.5758	1.0542	.5289	.8809	.8886	.8682	1.0042	.8903	1.0175
8	1.2476	1.4137	.6512				1.4709	.9883	1.1142
9	2.6271	1.8957	.8018						
10									
Intercept		.1352	.1233		.5290	.4117		.4287	.5389
Slope		.2934	.2080		.0741	.1066		.1044	.0908

CHAPTER 6

An assessment of the influence of host generated immunity on survival and fecundity.

The phenomenon of age dependent survival and fecundity in parasitic organisms on, or in, their hosts has been ascribed to two causes, host generated immune responses and senescence (chapter 11a).

In this chapter two approaches to the assessment of the influence of host generated immune responses are described. In section (a) challenge infections are compared with primary infections to determine whether survival and fecundity are reduced. In section (b) flukes transplanted onto naive hosts are compared with those remaining on the original host throughout the period of infection in an attempt to determine the effect on survival of transfer to those naive hosts.

a) Primary and challenge infections

i) Survival

The observed survival characteristics of T. patialense infections on B.rerio in the 28-32 mm length class at 23°C appear to be similar in primary and challenge infections. This is so both for a 14 fluke initial parasite density and for hosts exposed to 370 cercariae giving an average initial parasite density of 145.8 flukes per host in the primary infections, and 132.7 in the challenge infections (fig. 40A, B; tables 26, 29).

The instantaneous mortality for the challenge infections was determined (equation 1) and the relationship between death rate and time described empirically using the exponential model (equation 2). Table 28 shows the observed and calculated instantaneous death rates at a series of consecutive points in time.

Using the survival model (equation 4) the predicted survival curves were obtained (table 29). These showed a good fit to the

FIG.40.

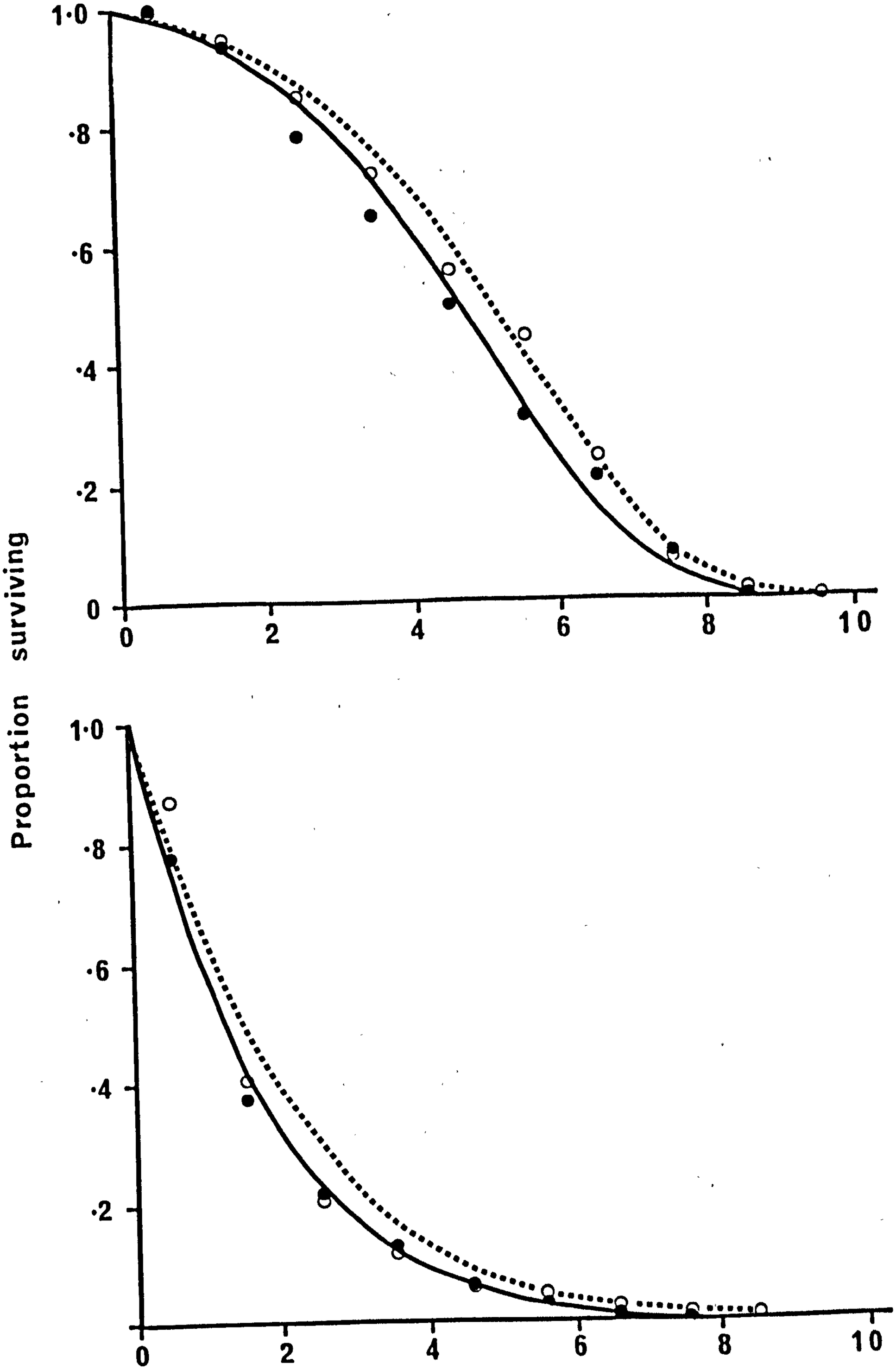
Fig. 40

The mean proportion of parasites surviving at successive weekly midpoints for primary and secondary infections at 23°C.

1. Open circles denote observed survival for primary infections.
2. Dashed lines represent the curves predicted for the primary infections by an empirical survival model (equation 4).
3. The solid circles denote observed survival for the challenge infections.
4. The solid lines represent the curves predicted for the challenge infections by an empirical survival model (equation 4).

A. Initial parasite density 14 flukes per host.

B. Hosts exposed to 370 cercariae giving average infection levels of 145.8 flukes per host (primary infections) and 132.7 (challenge infections).


Proportion surviving
1·0
·8
·6
·4
·2
0
0
2
4
6
8
10
Weeks

observed points and a close similarity between the curves for primary and challenge infections at both parasite densities was apparent (fig. 40A, B).

The instantaneous death rates were transformed into their natural logarithms in order to fit linear regressions and obtain constants a (intercept) and b (slope) for the exponential models. An analysis of covariance was carried out (LeCren, 1951; Snedecor and Cochran, 1967) to compare the regressions for the instantaneous death rates for the primary and challenge infections at each density.

The natural logarithms of the observed points for the primary and challenge infections and the regressions for both density levels are shown in figure 41 A, B. The analysis of variance (tables 32, 33) showed that there were no significant differences between either the slopes or the intercepts of the regressions at either density level ($P > .10$ in each case).

ii) Fecundity

The egg production of the challenge infection at the 14 fluke per host level was not determined.

From fig. 42 and tables 30 and 31 it is clear that egg production in the primary infections and challenge infections at initial parasite densities of 145.6 and 132.7 parasites per host respectively do not differ in terms of the rate per surviving fluke. These results are discussed in chapter 11a, c.

b) Survival of flukes transplanted to previously uninfected fish

Flukes were removed from their hosts seven days post infection and transplanted onto previously uninfected fish, six hosts were given infections of 3, 4, 7, 8, 11 and 12 flukes.

From fig. 43 it appears that the proportion of flukes surviving at a series of consecutive points in time in this transplant experiment is similar to that from the original age dependent survival

FIG.41.

Fig. 41

Natural logarithms of the instantaneous death rates (equation 1) of parasites at a series of consecutive points in time.

1. The open circles denote the observed points for primary infections.
2. The dashed lines are regressions fitted using a linear least squares technique to the observed data for the primary infections.
3. The solid circles denote the observed points for challenge infections.
4. The solid lines are regressions fitted to the observed data for the challenge infections.

A. Initial parasite density of 14 flukes per host

B. Hosts exposed to 370 cercariae giving average infections of 145.8 flukes per host (primary infections) and 132.7 flukes per host (challenge infections).

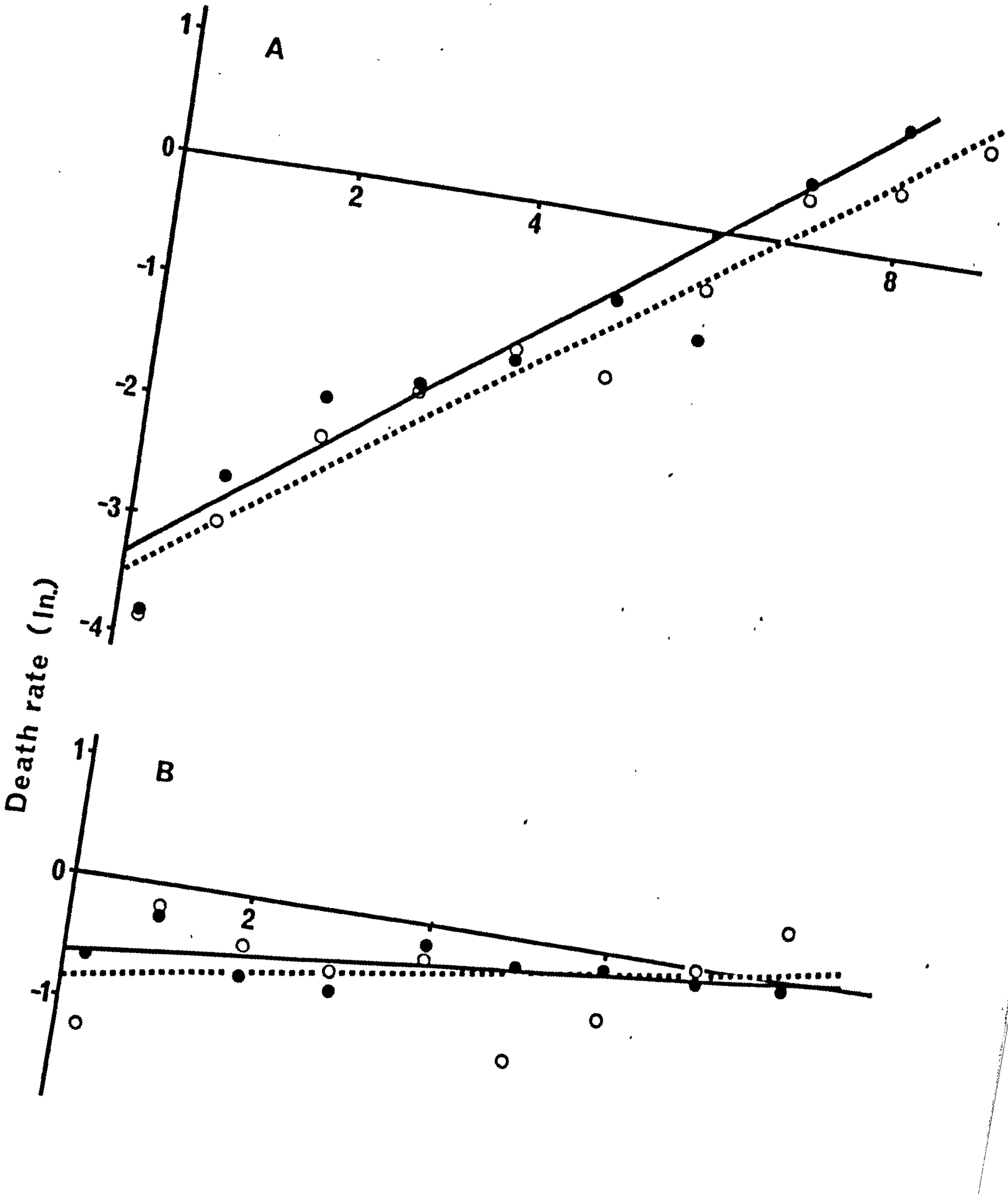

A
1
0
-1
-2
-3
-4
2
4
8
B
1
0
-1
2
Death rate (ln.)
Weeks

FIG.42.

Fig. 42

Egg production per surviving fluke per hour against time at 23°C.

1. The dashed line and open circles denote egg production in the primary infections (mean initial parasite density 145.8 flukes per host).
2. The solid line and solid circles denote egg production in the challenge infections (mean initial parasite density 132.7 flukes per host).
3. The vertical lines denote the 95% confidence limits round the observed points.

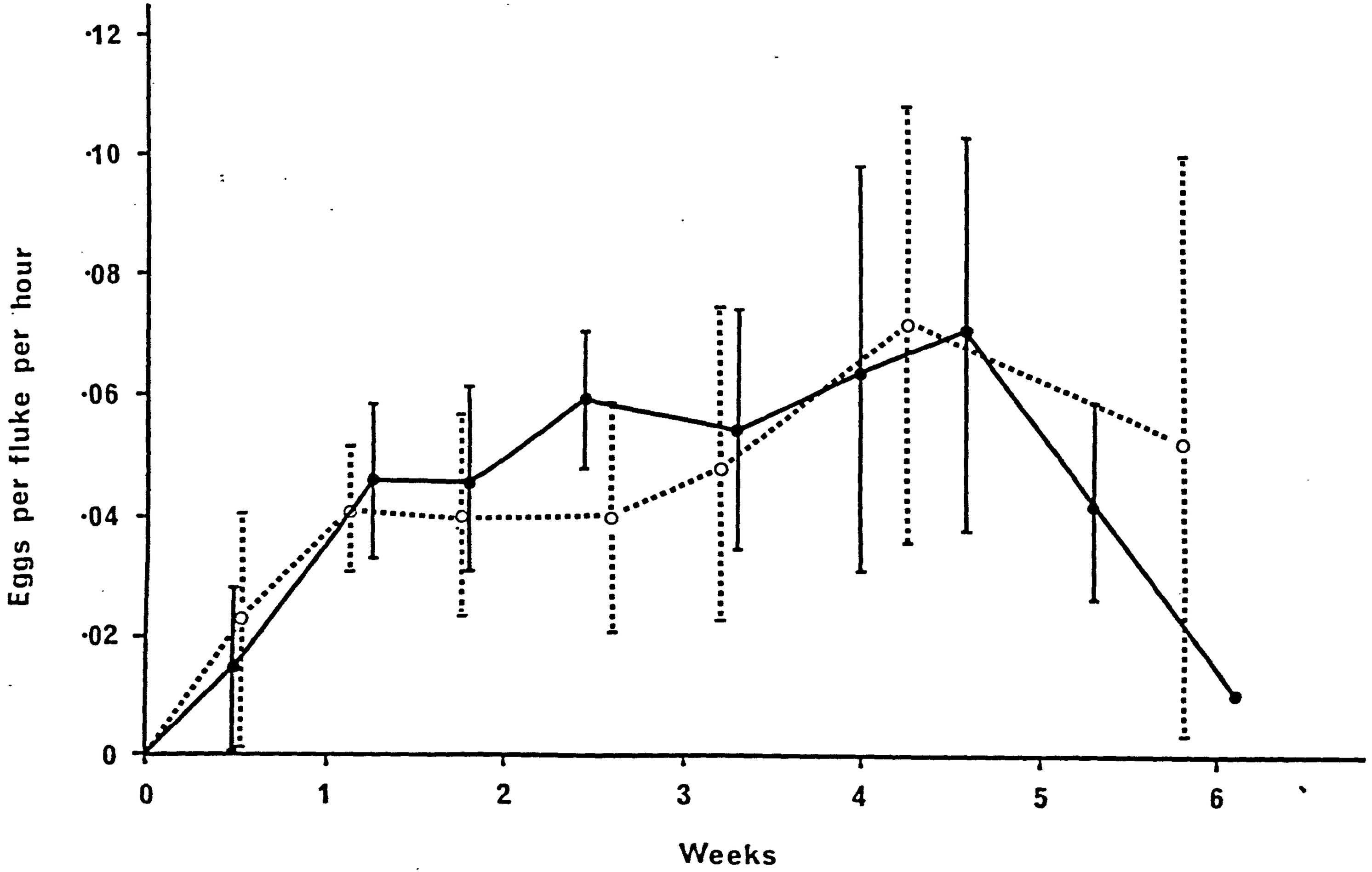
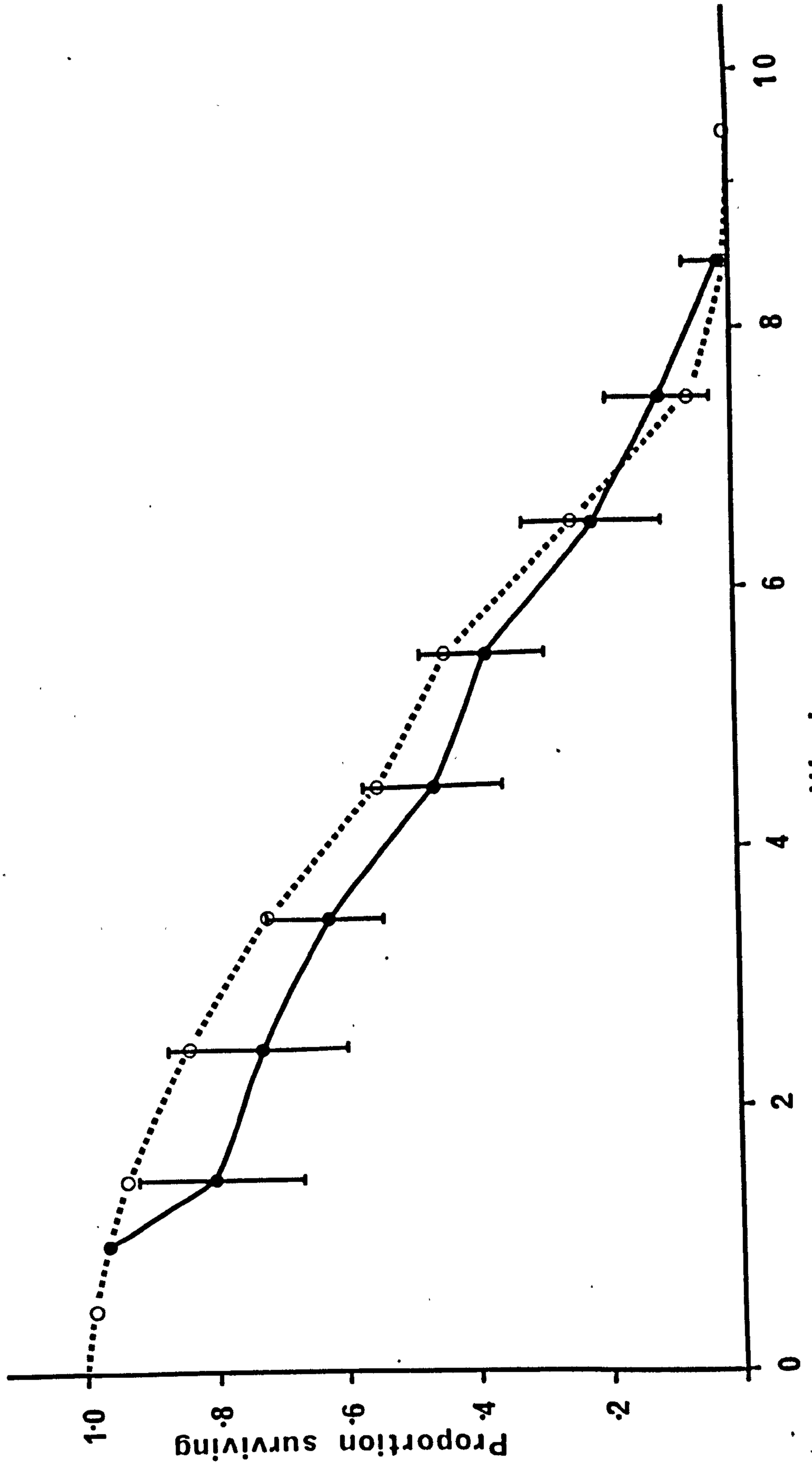

Eggs per fluke per hour
·12
·10
·08
·06
·04
·02
0
0
1
2
3
4
5
6
Weeks

FIG.43.

Fig. 43

The proportion of flukes surviving at a series of consecutive points in time at 23°C.

1. The solid circles and solid line shows the results for transplanted flukes.
2. The dashed line and open circles show the results for the original age dependent survival experiment with 14 flukes per host initial parasite density at 23°C.
3. The vertical bars denote the 95% confidence limits for the transplantation experiment. For the confidence limits for the original age dependent experiment, fig. 9.


Proportion surviving
1·0
·8
·6
·4
·2
0
2
4
6
8
10
Weeks

experiment. The only obvious difference is a sharp drop in the first half week post-transplantation. This initial drop suggests that some flukes were damaged during transplantation or failed to adapt to the microenvironment of their new host.

From the adjusted survival data for the transplant experiment (table 34A) the instantaneous death rates for the flukes were determined at a series of consecutive points in time (equation 1) (table 34B). The natural logarithms of this data were then compared with those for the original age dependent data (table 28) to see if the linear regression for the sets of data were significantly different using the analysis of variance described in section a. The ln. transformed data and the regression lines for the original age dependent and the transplant experiments are shown in fig. 44. There was no significant difference between either the slopes ($P > .10$) or intercepts ($P > .10$) of the regressions (table 35).

Thus, despite the fall in the proportion of flukes surviving in the first half week post-transplantation, transplantation cannot be shown to have had any significant effect on the survival of T.patialense.

Using the coefficients a (intercept) and b (slope) from the control experiment the instantaneous death rate predicted by equation 2 was determined for a series of consecutive points in time. It can be seen from table 34B that these are in good agreement with the observed data.

FIG.44.

Fig. 44

Natural logarithmic transformations of the instantaneous death rates (equation 1) of flukes at a series of consecutive points in time.

1. The solid circles show the observed results for transplanted flukes.
2. The solid line is a linear least squares regression (equation 21) fitted to the above results.
3. The open circles show the observed results for the age dependent survival experiment (chapter 3).
4. The dashed line is a linear least squares regression (equation 21) fitted to the above results.

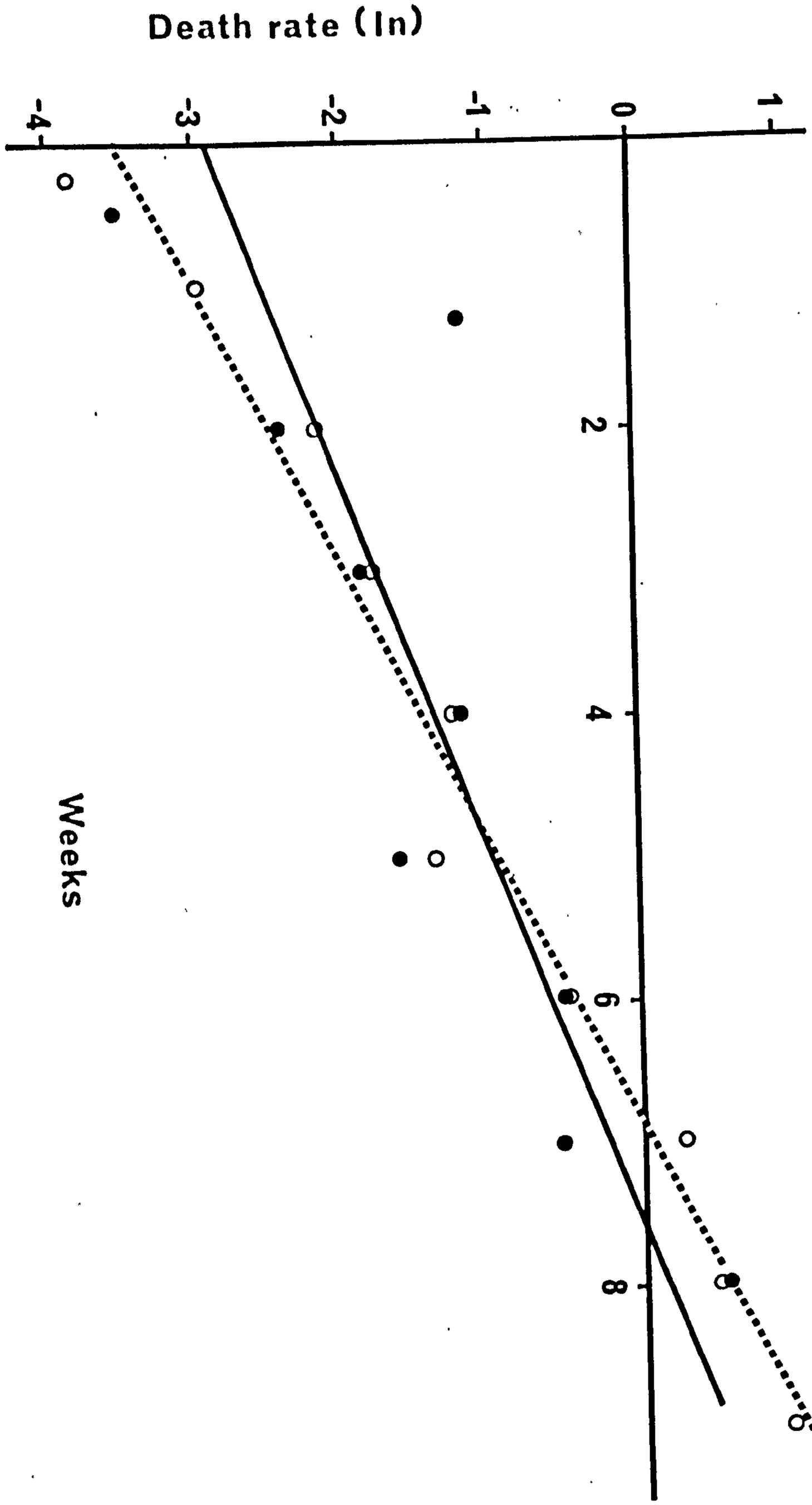

Death rate (ln)
-4
-3
-2
-1
0
1
2
4
6
8
Weeks

Table 28 Death rates in primary and secondary infections.

Time (weeks)	Primary 14 fluke infection, ln. of instantaneous death rate.	14 fluke challenge infection			Primary 145.6 fluke infection ln. of instantaneous death rate.	132.7 fluke challenge infection		
		observed instantaneous death rate.	calculated rate from exponential model.	ln. of observed instantaneous death rate.		observed instantaneous death rate.	calculated death rate from exponential model.	ln. of observed instantaneous death rate.
.25	-3.8632	.0022	.0385	-3.8167	-1.2375	.5124	.5389	-.6686
1	-2.9604	.0758	.0569	-2.5797	- .2146	.7572	.5697	-.2781
2	-2.1742	.1702	.0966	-1.7708	- .3905	.5191	.6135	-.6557
3	-1.8502	.1890	.1640	-1.6660	- .4927	.5155	.6607	-.6626
4	-1.3067	.2637	.2783	-1.3329	- .3061	.8185	.7115	-.2003
5	-1.4939	.4939	.4725	- .7054	-1.0153	.7604	.7662	-.2739
6	- .5329	.3945	.8020	- .9301	- .5729	.8740	.8252	-.1347
7	.2766	1.6662	1.3613	.5105	- .0042	.8809	.8886	-.1268
8	.4757	2.7672	2.3107	1.0178	- .3859			
9	.9439							
Slope (b)	.5120		.5272	.5272	.1044		.0741	.0741
Inter-cept (a)	-3.219		.0340	-3.376	- .8470		.5290	-.6368

Table 29 The mean observed proportions of parasites surviving in challenge infections at successive weekly mid-points and the proportions calculated from the exponential survival model.

	14 fluke challenge infection		132.7 fluke challenge infection	
Time (weeks)	observed	calculated	observed	calculated
0.5	.9989	.9810	.747	.764
1.5	.9260	.9261	.363	.432
2.5	.7811	.8399	.216	.234
3.5	.6466	.7115	.129	.121
4.5	.4967	.5369	.0569	.0593
5.5	.3031	.3329	.0266	.0275
6.5	.2043	.1479	.0111	.0121
7.5	.0780	.0373	.0046	.0050
8.5	.0049	.0036		
9.5				

Table 30 Egg output per fluke per hour for primary infections (average initial density 145.8 parasites per host).

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-5	0.57	.0232	.0235	6	.0246
6-10	1.17	.0410	.0157	10	.0113
11-15	1.76	.0397	.0256	11	.0172
16-20	2.56	.0397	.0245	9	.0188
21-25	3.24	.0480	.0246	6	.0259
26-35	4.25	.072	.0359	6	.0366
36-45	5.857	.052	.0396	4	.0490

Table 31 Egg output per fluke per hour for challenge infections (average initial density 132.7 parasites per host).

Time interval (days)	Mean time (weeks)	Eggs per fluke per hour.	Standard deviation	Number of samples	95% confidence limits
1-5	.51	.0144	.0191	7	.0149
6-10	1.24	.0462	.0126	6	.0132
11-15	1.80	.0455	.0164	7	.0152
16-20	2.44	.0595	.0125	7	.0116
21-25	3.30	.0537	.0235	8	.0196
26-30	4.06	.0637	.0369	7	.0341
31-35	4.64	.0706	.0210	4	.0334
36-40	5.29	.0418	.0171	7	.0158
41-45	6.14	.0104	.0147	2	-

Table 32 Analysis of covariance to compare the slopes and intercepts of ln. transformations of the instantaneous death rates of the 145.8 fluke per host infections and the 132.7 fluke per host reinfections.

	df	Σx^2	Σxy	Σy^2	Regression coefficient	Deviations from regression		
						df	SS	Ms
Within	8	58.0556	6.0599	1.9364	.1044	7	1.3039	.18627
Primary Reinfection	7	40.3047	2.9852	.41711	.07407	6	.1960	.03267
						13	1.4999	.11538
Pooled (W)	15	98.3603	9.0451	2.3535		14	1.5217	.1087
				Difference between slopes		1	.0218	.0218
Between (B)	1	1.0441	- .1084	.0113				
W + B	16	99.4044	8.9367	2.3648		15	1.56137	
				Between adjusted means			.03967	

Comparison of slopes F = .0218 / .21894 = .0996 N.S. (P>.10)

Comparison of intercepts F = .03967/ .1087 = .3649 N.S. (P>.10)

Table 33 Analysis of covariance to compare the slopes and intercepts of ln. transformations of the instantaneous death rates of the 14 flukes at 23°C experiment (chapter 3) and the 14 fluke per host reinfection experiment.

	df	Σx^2	Σxy	Σy^2	Regression coefficient	Deviations from regressions		
						df	SS	MS
Within	9	80.3065	41.013	21.666	.5108	8	.070727	.08841
Primary Reinfection	8	58.0556	30.6096	17.4484	.5272	7	1.3096	.1871
						15	2.01687	.13446
Pooled (W)	17	138.3621	71.6226	39.1144	.5176	16	2.0392	.12745
				Difference between slopes		1	.02233	.02233
Between (B)	1	1.1708	.0092	- .0003				
W + B	8	139.5329	71.6318	39.1141		17	2.3406	.13768
				Between adjusted means		1	0.3014	0.3014

Comparison of slopes F = .02233/.13446 = .16607 N.S. (P >.05)

Comparison of intercepts F = .3014/.12775 = 2.3648 N.S. (P >.05)

Table 34 A. Proportions of flukes surviving after transplantation at a series of consecutive points in time.
B. Instantaneous death rates at a series of consecutive points in time.

A					B			
Time (weeks)	Proportion of transplanted flukes surviving.	Times the proportion surviving at one week at 14 flukes per host.	Standard deviation	95% confidence limits	Time (weeks)	Instantaneous death rate.	ln. of instantaneous death rate.	Instantaneous death rate predicted by exponential model.
0.5					0.5	.0305	-3.4900	.0667
1.0	1.0000	.9700			1.25	.3164	-1.1507	.0887
1.5	.8281	.8032	.1280	.1343	2	.0883	-2.4270	.1181
2.5	.7581	.7354	.1333	.1396	3	.1542	-1.8695	.1729
3.5	.6498	.6303	.0901	.0945	4	.3025	-1.1957	.2531
4.5	.4802	.4658	.1057	.1109	5	.1910	-1.6555	.3706
5.5	.3967	.3848	.0936	.0982	6	.5973	- .5153	.5426
6.5	.2183	.2118	.1150	.1207	7	.5688	- .5642	.7944
7.5	.1236	.1200	.0940	.0987	8	1.7869	.5805	1.1630
8.5	.0207	.0201	.0496	.0521				
Slope (b)							.3812	.3812
Intercept (a)							-2.898	.0551

Table 35 Analysis of covariance to compare the slopes and intercepts of ln. transformations of the instantaneous death rates of the 14 flukes at 23°C experiment (chapter 3) and the transplantation experiment.

	df	Σx^2	Σxy^2	Σy^2	Regression coefficient	Deviations from regression df	SS	MS
Within Normal Transplant	9	80.3065	41.013	21.666	.5108	8	.70727	.08841
	8	54.5025	20.7768	11.2052	.3312	7	3.2849	.46927
						15	3.99217	.26614
Pooled (W)	17	134.809	61.7898	32.8712	.45835	16	4.5498	.28436
				Difference between slopes		1	.55763	.55763
Between (B)	1	.9238	.2426	.0614				
W + B	18	135.7328	62.0324	32.9326		17	4.58264	.26957
				Between adjusted means		1	0.03284	0.03284

Comparison of slopes F = .55763/.26614 = 2.09526 N.S. (P<.10)

Comparison of intercepts F = .03284/.28436 = .11549 N.S. (P<.10)

CHAPTER 7

The effect of host size on survival at 23°C

In the 28.1-32 mm fish host size class, and the class containing fish more than 32 mm in length, the proportion of parasites surviving to the mid-point of each successive week post infection, show no obvious difference. It is clear, however, that survival progressively diminishes in the smaller host size classes (fig. 45, table 36). In the smallest size class, no parasites survive to the mid-point of the first week post infection.

The average time taken for the number of parasites to fall to half the original level in each host size class, also shows little difference between the two largest size classes. In progressively smaller fish, however, this time rapidly decreases (fig. 46, table 37).

The mean size of the hosts, in all size classes except one, increased appreciably between the time of infection and both the time to 50% parasite survival, and the time when the last parasite had died. The exception was in the 8.1-12.0 mm size class (fig. 47, table 37). These increases in the size of the hosts during the experiments, generally conformed to the growth curve for B.rerio obtained in the laboratory, which exhibited a gradual decrease in the growth rate with increasing size (fig. 48).

The underlying relationship between parasite survival, host size and host growth rate is complex, and could only be examined in detail by means of large scale factorial experiments.

FIG.45.

Fig. 45

The proportions of parasites surviving at the midpoints of successive weeks post infection for six size classes of fish hosts.

1. The solid circles and solid lines denote the observed results.
2. The heavily dashed lines link each time point between different size classes.

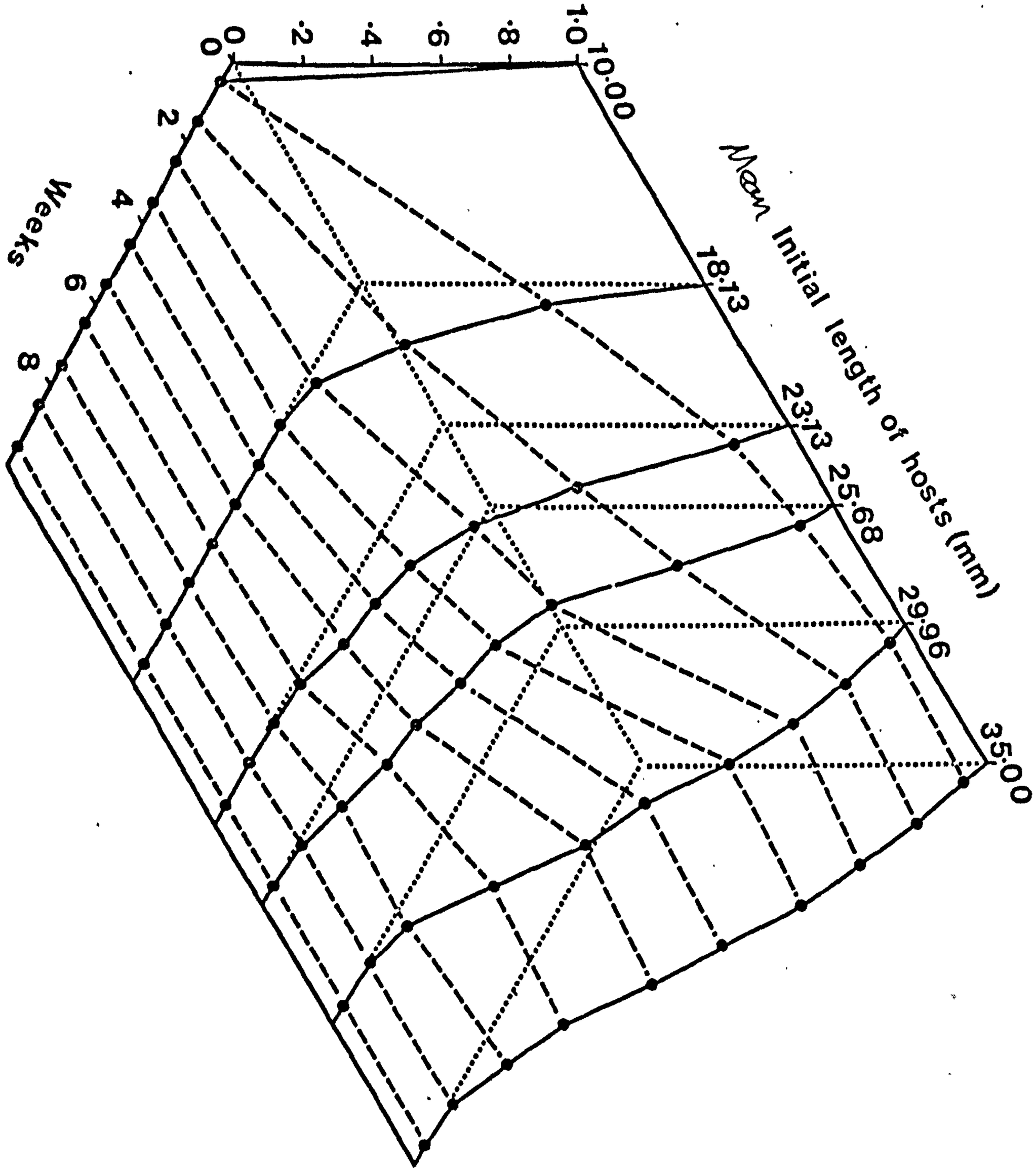

Proportion of flukes surving
0
·2
·4
·6
·8
1·0
10·00
18·13
23·73
25·68
29·96
35·00
Mean Initial length of hosts (mm)
Weeks
2
4
6
8

FIG.46.

Fig. 46

The average time for the number of parasites to fall to 50% of their initial numbers against the mean lengths of each length class of fish hosts at the time of infection.

1. The vertical bars denote the 95% confidence limits.

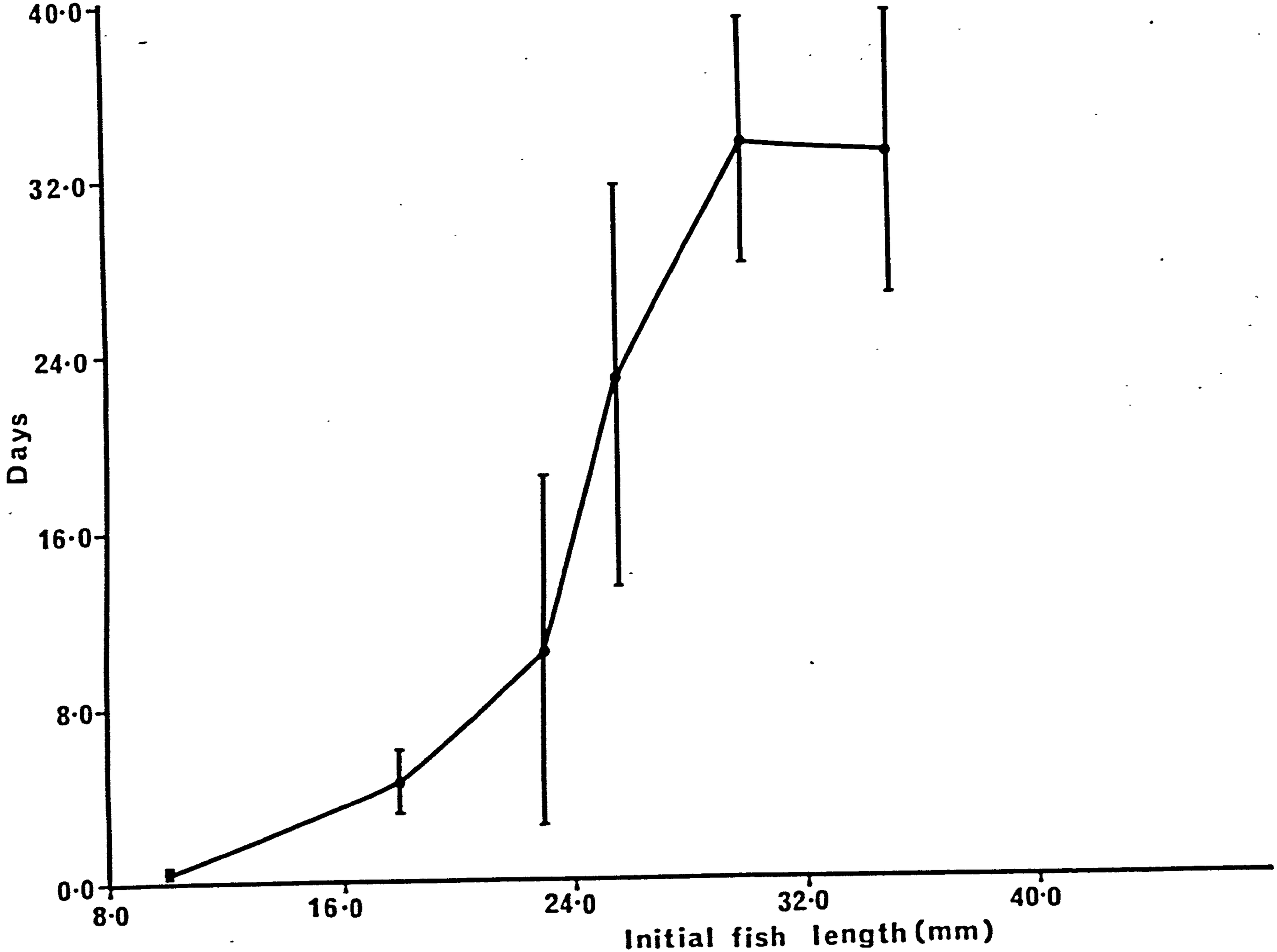

40·0
32·0
24·0
16·0
8·0
0·0
Days
8·0
16·0
24·0
32·0
40·0
Initial fish length (mm)

FIG.47.

Fig. 47

The increase in the mean size of the hosts in each size class against the percentage of parasites surviving.

1. For the 95% confidence limits see table 37.

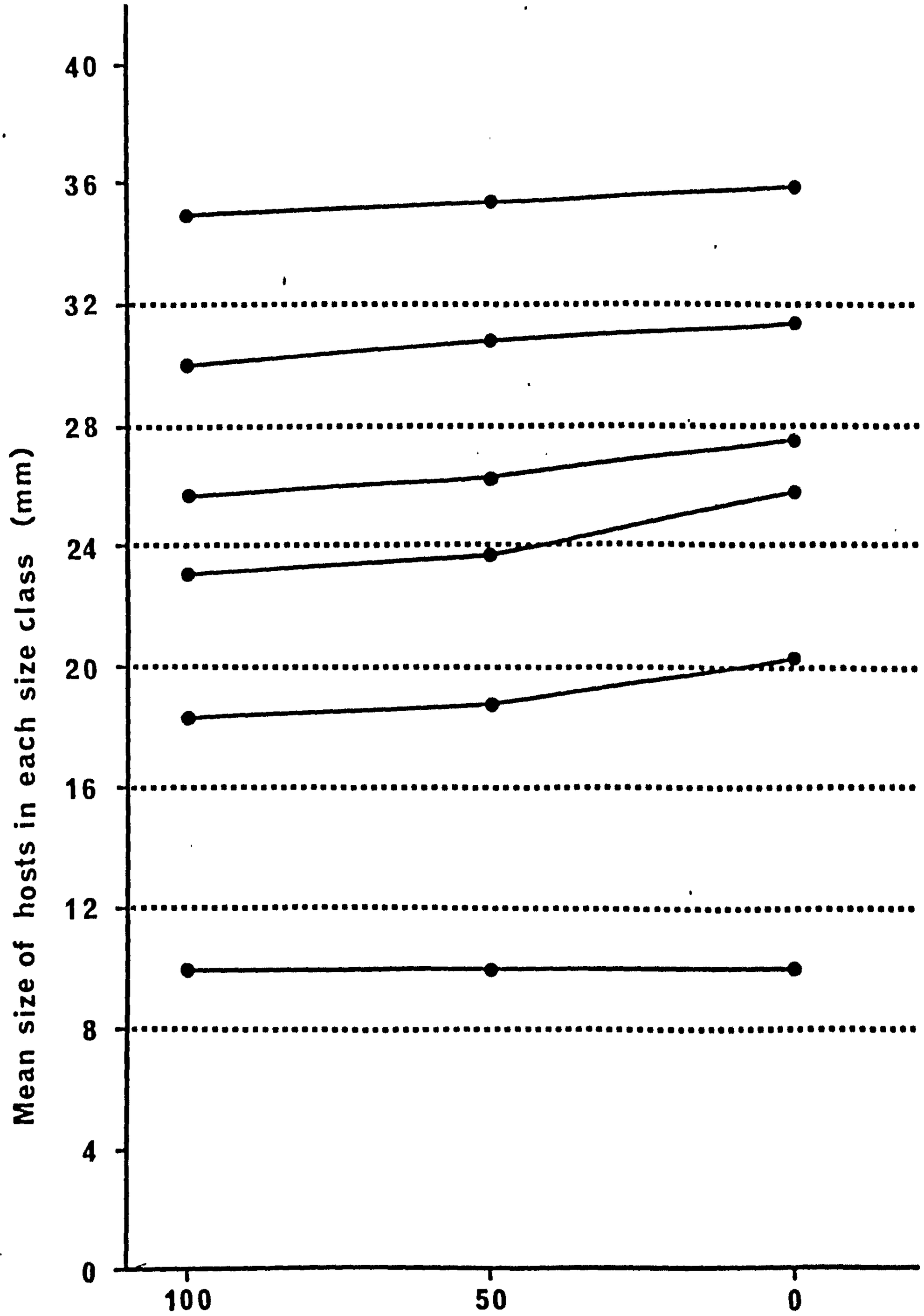

40
36
32
28
24
20
16
12
8
4
0
Mean size of hosts in each size class (mm)
100
50
0
Percentage of parasites surviving

FIG.48.

Fig. 48

Increase in the length of uninfected B.rerio with time.

1. The solid circles are the means of sets of observed points.
2. The vertical bars denote the 95% confidence limits.

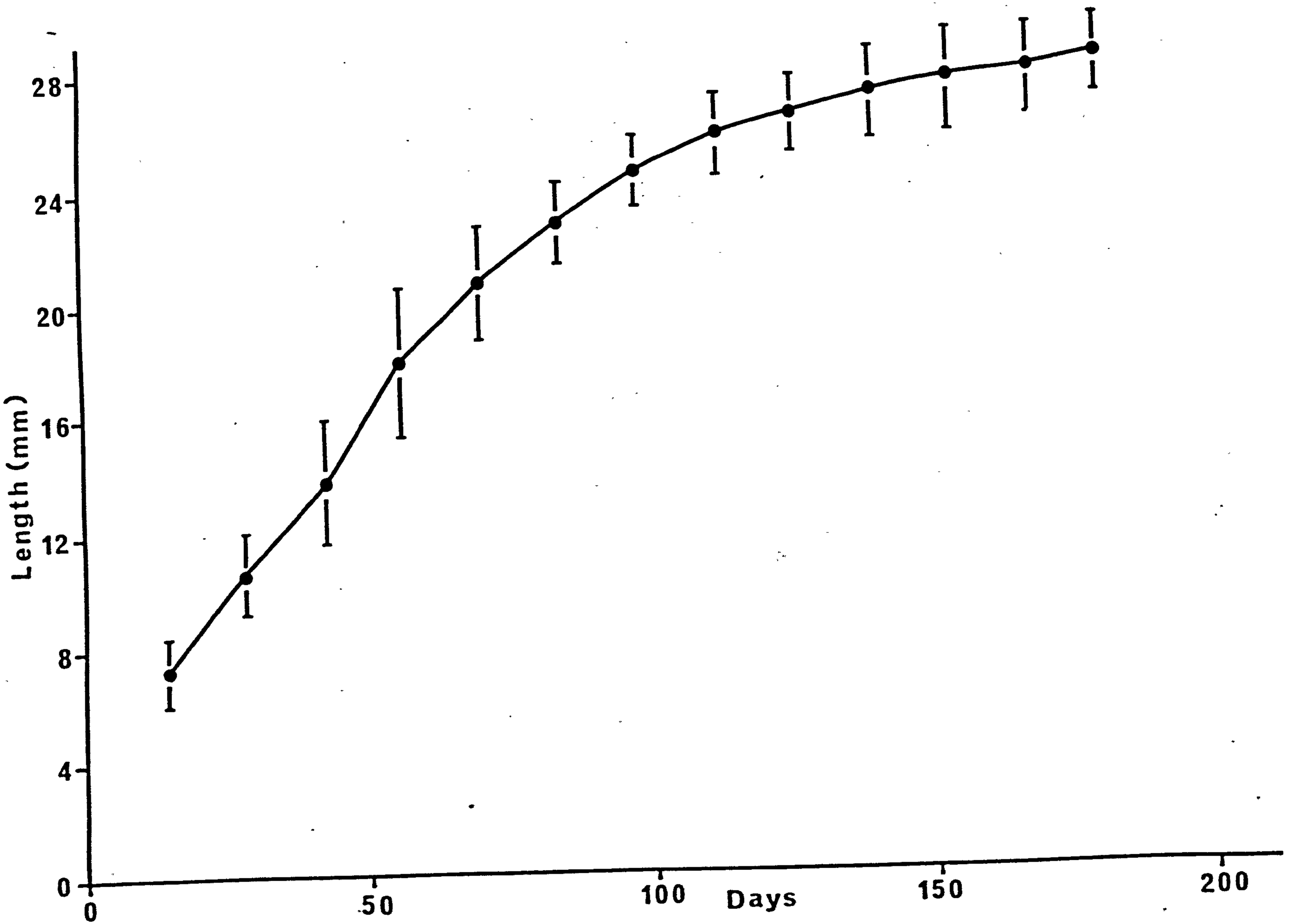

Length (mm)
28
24
20
16
12
8
4
0
0
50
100
Days
150
200

Table 36 Survival of T.patialense on different sized hosts.

	8.1 - 12 mm (4 hosts)		16.1 - 20 mm (4 hosts)		20.1 - 24 mm (4 hosts)		24.1 - 28 mm (11 hosts)		28.1 - 32 mm (8 hosts)		32+ mm (5 hosts)	
Time (wks.)	proportion surviving	95% c.l.	proportion surviving	95% c.l.	proportion surviving	95% c.l.	proportion surviving	95% c.l.	proportion surviving	95% c.l.	proportion surviving	95% c.l.
0	1.000		1.000		1.000		1.000		1.000		1.000	
0.5	0		.570	.105	.875	.320	.929	.066	.997	.007	.966	.060
1.5			.220	.238	.488	.570	.646	.111	.930	.043	.910	.048
2.5			.035	.065	.250	.190	.348	.156	.846	.081	.805	.145
3.5	0		.138	.208	.256		.184	.726	.136	.702	.149	
4.5			.105	.194	.213		.194	.539	.157	.536	.203	
5.5			.075	.139	.156		.174	.439	.142	.400	.151	
6.5			.015	.048	.133		.151	.237	.147	.208	.144	
7.5			.006	.020	.069		.090	.051	.051	.118	.083	
8.5			0		.018		.033	.010	.025	.016	.038	
9.5					.001		.002	0		0		
10.5					0							

Table 37 Mean size of fish in each size class and mean times to 100%, 50% and 0% of the initial number of flukes.

No. of hosts	Size class	100% of initial infection surviving.				50% of initial infection surviving.				0% of initial infection surviving.			
		Size		Days		Size		Days		Size		Days	
		(mm)	95%c.l.		95%c.l.	(mm)	95%c.l.		95%c.l.	(mm)	95%c.l.		95%c.l.
4	8.1-12.0	10.00	0.65	0		10.00	0.65	0.58	0.16	10.00	0.65	2.00	0.00
4	16.1-20.0	18.13	0.96	0		18.79	0.68	4.58	1.56	20.31	1.72	16.75	8.30
4	20.1-24.0	23.13	2.29	0		23.70	1.68	10.31	7.88	25.86	4.09	33.25	28.23
11	24.1-28.0	25.68	0.64	0		26.38	0.79	22.60	9.06	27.47	1.08	36.40	12.01
8	28.1-32.0	29.96	0.96	0		30.81	1.06	33.64	6.20	31.42	1.16	55.00	6.45
5	32+	35.00	2.00	0		35.47	1.79	33.00	7.13	35.8	1.59	56.5	6.12

CHAPTER 8.

Factors affecting the rate of egg production

Although this thesis is mainly concerned with population studies, growth of the parasite on the fish host has been examined in some detail. These studies had two main objectives; firstly, it was hoped that such studies might reveal density dependent growth effects. If present, these could account for the reduced rate of egg production at high initial parasite densities. Secondly, the investigations were regarded as an attempt to correlate the growth of the vitelline glands with the commencement of egg production and the subsequent rapid rise in egg production per surviving fluke (fig. 10).

In addition to these experiments an attempt was made to correlate the occurrence of certain reproductive abnormalities in adult T.patialense with the declining phase of egg production and the mode of feeding of adult T.patialense on the fish host was examined in a preliminary way.

a) Growth

i) Growth in width of the adult parasite at three initial parasite densities.

A number of Brachydanio rerio were infected with either 14, 30 or an average of 124 flukes per host and maintained at 23°C. At weekly intervals numbers of flukes were removed from some of the hosts and their widths determined. The initial widths at the time of infection were determined by measuring the widths of cercariae.

Due to the ease with which flukes can be deformed the measuring technique, which involved flattening batches of flukes under coverslips, could have led to serious inaccuracies in the results. In an attempt to estimate the extent of variability produced in this way control experiments were carried out. No significant difference

between the widths of flukes on six separate slides in either of the two control experiments was observed (methods 24b) (analysis of variance; see Bailey, 1959) using flukes from infected hosts seven days post infection ($P > .10$ in each case). Thus the measuring technique did not tend to introduce a significant increase in variability between slide preparations.

The growth curves in figure 49 (table 39) show that at initial parasite densities of 14 and 30 flukes per host growth is markedly age dependent with a sharp increase in width in the first week post infection followed by a fall in the rate of increase. A virtual cessation of growth is indicated by the fourth week post infection. Similarly, the instantaneous growth rates (table 38) calculated from the growth data using the formula

$$\ln \text{width}_{(t+1)} - \ln \text{width}_{t} \quad (27)$$

(Ricker, 1975) show a steady decrease in time.

At the average initial infection level of 124 flukes per host there is evidence that growth is reduced. After two weeks the average width of flukes at this density had increased from .638 to .837 mm whilst there were increases to .939 and .915 at 14 and 30 flukes per host respectively at this stage.

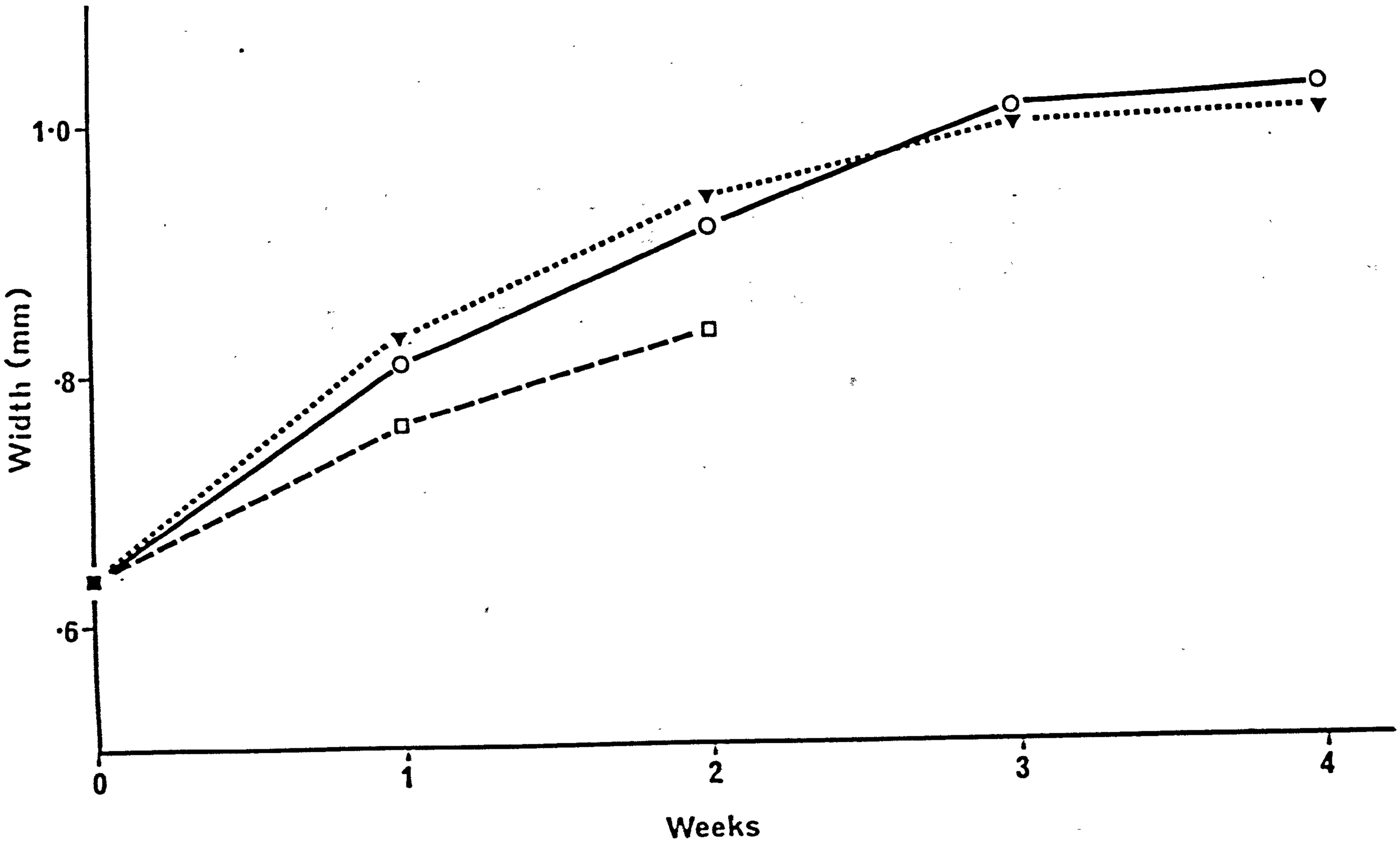
The means of the sets of data for fluke widths two weeks post infection at the three initial parasite densities were compared using a t-test for the comparison of small samples where the variances are assumed to be equal (Bailey, 1959). There was found to be no significant difference between the means at 14 and 30 flukes per host levels ($P > .10$). The means of the 14 and 30 fluke level infections both differed significantly from that at the 124 level ($P < .01$ and $< .001$ respectively). The highest parasite level therefore, causes a significant reduction in the size of flukes at two weeks post infection.

FIG.49.

Fig. 49

The mean widths of T.patialense at a series of consecutive points in time post infection.

1. The solid square denotes the mean width at the time of infection i.e. the cercarial width.
2. The solid triangles denote the mean widths of adult flukes with an initial parasite density of 14 flukes per host.
3. The open circles denote the mean widths of adult flukes with an initial parasite density of 30 flukes per host.
4. The open squares denote the mean widths of adult flukes with an average initial parasite density of 124 flukes per host.


1·0
·8
·6
Width (mm)
0
1
2
3
4
Weeks

No attempt could be made to determine mean parasite widths after the second week post infection at the 124 fluke per host level. This was due to the high mortality of flukes at the high initial parasite density (chapter 5) leaving so few flukes after this time that an impossibly large number of replicates would have been required to obtain sufficient data, given the available supply of cercariae of T.patialense. This is unfortunate as it would have been interesting to see if the recovery in egg output (fig. 33F) was associated with an increase in size.

A variety of models exist for fitting growth data. One of the most versatile of thise is the Compertz function (Compertz, 1825; D'Arcy-Thompson, 1917) which has been utilised in a wide variety of biological situations, for example, in the analysis of growth of clams (Weymouth, McMillan and Rich, 1931), human populations (Shryock and Siegel, 1973) and tumours (Norton, Simon, Brereton and Bogdon, 1976). Sullivan (1968) compares the Compertz function favourably with the von Bertalanffy growth model for fitting weights-at-age data for four fish species.

The Compertz function has the form:

$$Yt = Be^{a/b\left[1 - e^{bt}\right]} \qquad (28)$$

where Y is the size of the organism at time t

;constant a is the intercept and constant b the slope from the linear regression fitted to the natural logarithms of the instantaneous growth rates.

;constant B is the size of the organism at birth i.e. $B = Y_o$

The model assumes that the rate of growth declines to zero as the animal ages and hence, has a fixed maximum size. From the shape of the observed curves (for example in fig. 49) this seems a reasonable assumption.

When t becomes large the model collapses giving

$$Y_\infty = Be^{a/b} \qquad (29)$$

where

Y_∞ is the maximum size attained.

The underlying model is

$$\frac{dy}{dt} = \lambda(t)\,Yt \qquad (30)$$

where

$\lambda(t)$ is the growth rate which is a function of age (t) and

$$\lambda(t) = ae^{-bt} \qquad (31)$$

This model of the instantaneous growth rate is clearly analogous with the model for the instantaneous death rate (equation 2). The constants a and b are again obtained from the linear regressions fitted to the natural logarithms of the observed instantaneous growth rates.

Table 38 shows the observed values for the instantaneous growth rates, the values predicted by equation 31, the values of coefficients a and b, and the correlation coefficients for the fit of the regressions for the three density classes. Table 39 and fig. 50 show the observed parasite widths and 95% confidence limits and the results predicted by the Gompertz function (equation 28).

The rather poor fit of the models to the data for both instantaneous growth rate (equation 31) (table38) and parasite width (equation 28) (table 39, fig. 51) at the 14 and 30 parasite per host density levels stems from the poor fits of the linear regressions to the natural logarithms of the observed instantaneous growth rates ($P > .05$ in each case that the correlation is insignificant). Due to this the resulting coefficients a and b introduce the inaccuracies into the models.

ii) <u>The development of the vitelline glands</u>.

The increases in the total areas of the flukes and of their

FIG.50.

Fig. 50

The observed mean widths of T.patialense at a series of consecutive points in time post infection and the widths predicted by a growth model.

1. The solid circles denote the observed points.
2. The short horizontal lines denote the 95% confidence limits for the observed points.
3. The solid lines denote the curves predicted by a Gompertz growth model (equation 28).

The values of the coefficients for the above model are given in table 38.

A. Initial parasite density 14 flukes per host

B. Initial parasite density 30 flukes per host

C. Average initial parasite density 124 flukes per host.

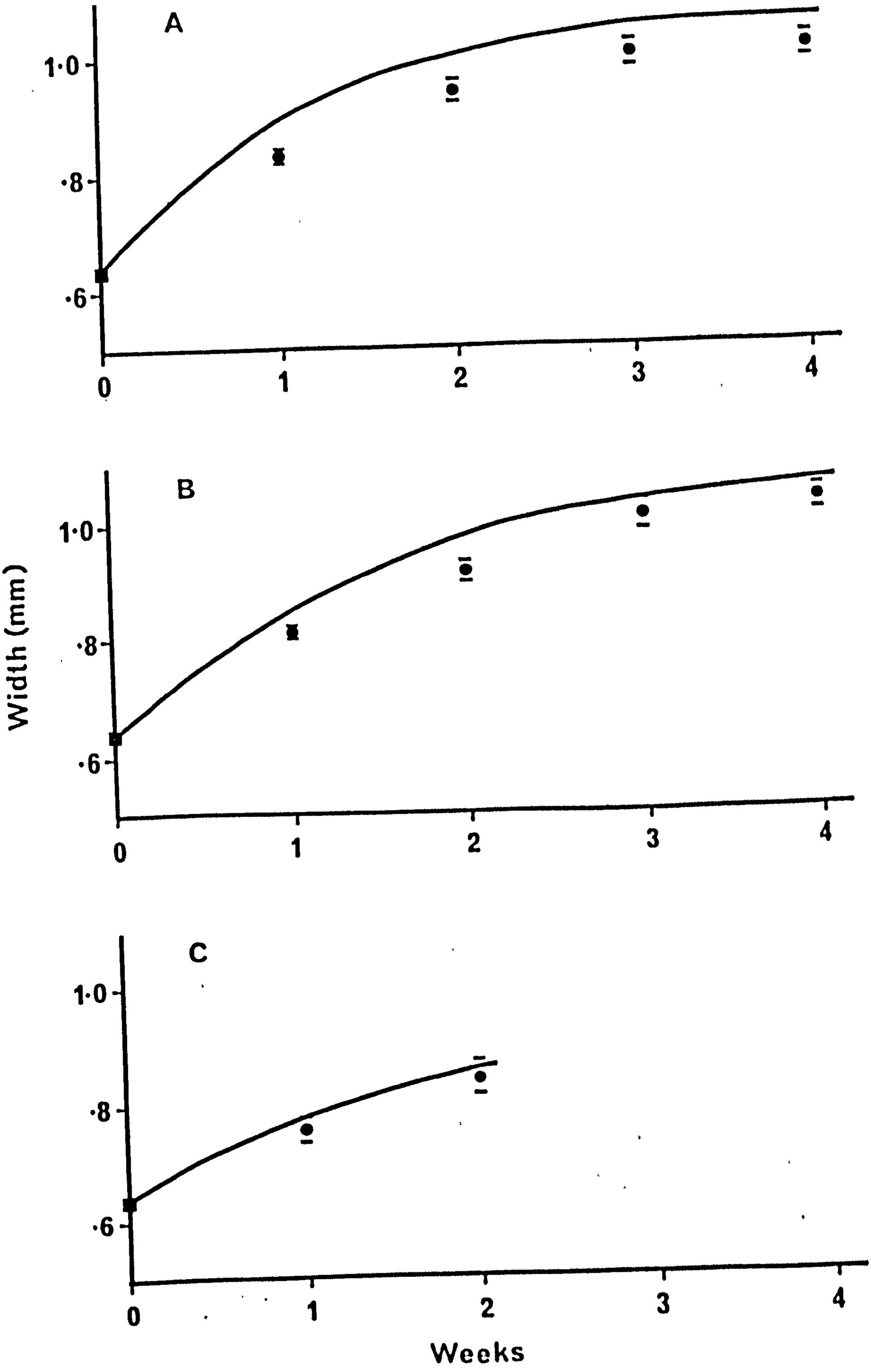

A
1·0
·8
·6
0
1
2
3
4
B
1·0
·8
·6
Width (mm)
0
1
2
3
4
C
1·0
·8
·6
0
1
2
3
4
Weeks

FIG.51.

Fig. 51

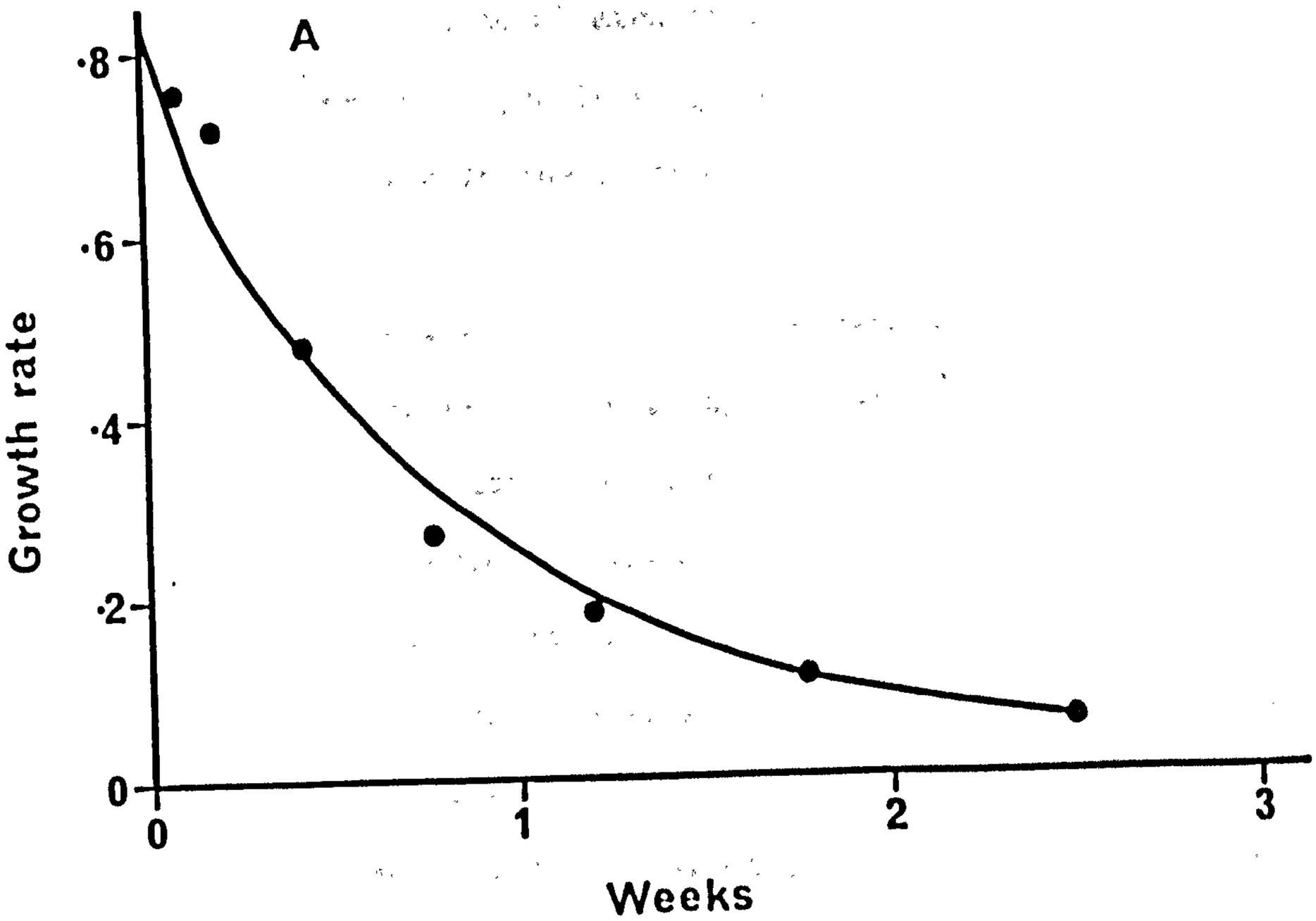
A. The instantaneous growth rate of the mean area of T.patialense at a series of consecutive points in time post infection.

1. The solid circles denote the observed points.
2. The solid line is the curve predicted by an exponential model (equation 31).

For the values of the coefficients for this model see table 40A.

B. The mean area of T.patialense at a series of consecutive points in time post infection.

1. The solid circles denote the means of sets of observed points.
2. The point at t=0 is the value at the time of infection and is the result for decaudated cercariae.
3. The vertical lines denote 95% confidence limits round the observed points.
4. The solid line denotes the curve predicted by a Gompertz growth model (equation 28) with the same coefficients as in A, above.


A
·8
·6
·4
·2
0
Growth rate
0
1
2
3
Weeks

B
30
24
18
12
Area (mm2)
0
1
2
3
Weeks

vitelline glands were determined utilising a photographic technique.

The increase in total area shows an initial steep rise which becomes increasingly gentle (fig. 51B, table 41B) and this is accompanied by a decline in the instantaneous growth rate of the area (fig. 51A, table 41A).

A linear regression fitted to the natural logarithms of the instantaneous growth rates of the areas provided the coefficients for the empirical models (equations 28 and 31) and in this case was a good fit ($P < .001$). The predicted curves for the instantaneous growth rate of the areas of the flukes (fig. 51A, table 41A) and the actual changing area of the flukes with time (fig. 51B, table 41B) fit the observed data extremely closely.

The observed area of the vitellaria increased with extreme rapidity to 23 times the original area in the first week post infection followed by smaller rises up to 2.85 weeks post infection (table 41B) and consequently the instantaneous growth rate drops steeply (table 40B). Figure 52 shows the extent of the vitelline glands in three flukes; A, at the time of infection; B, two days post infection and C, one week post infection.

A linear regression fitted to the natural logarithms of the instantaneous growth rates again provided the coefficients for equations 28 and 31. Although the regression is a significant fit to the data ($P < .01$) the predicted growth rate (table 40B) and area (table 42B) do not provide good fits to the observed data. To see if this was due to the extremely rapid initial drop in instantaneous growth rate, square root transformations of the area data were made (table 41D, fig. 53). The correlation coefficient for the fit of the linear regression to the transformed data was improved.

The curve predicted by the model for the instantaneous growth rate is shown in fig. 53C (table 40D) and for the square root

FIG.52.

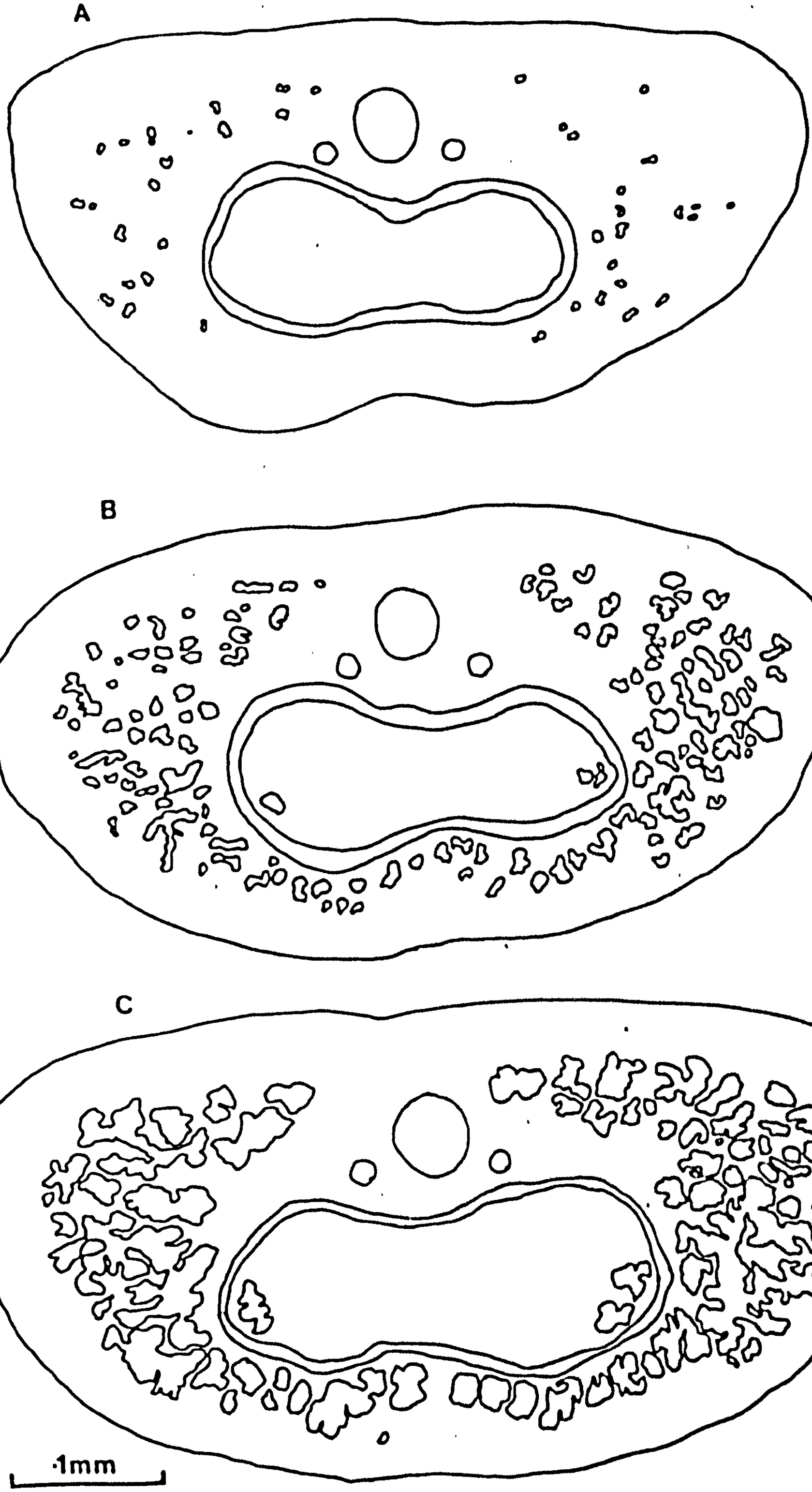
Fig. 52

The extent of the vitelline glands in three "typical" adult T.patialense.

A. At the time of infection.

B. Two days post infection.

C. One week post infection.


A
B
C
·1mm

FIGS.53,54.

Fig. 53

The instantaneous growth rate of the square root of the mean area of the vitelline glands of T.patialense at a series of consecutive points in time post infection.

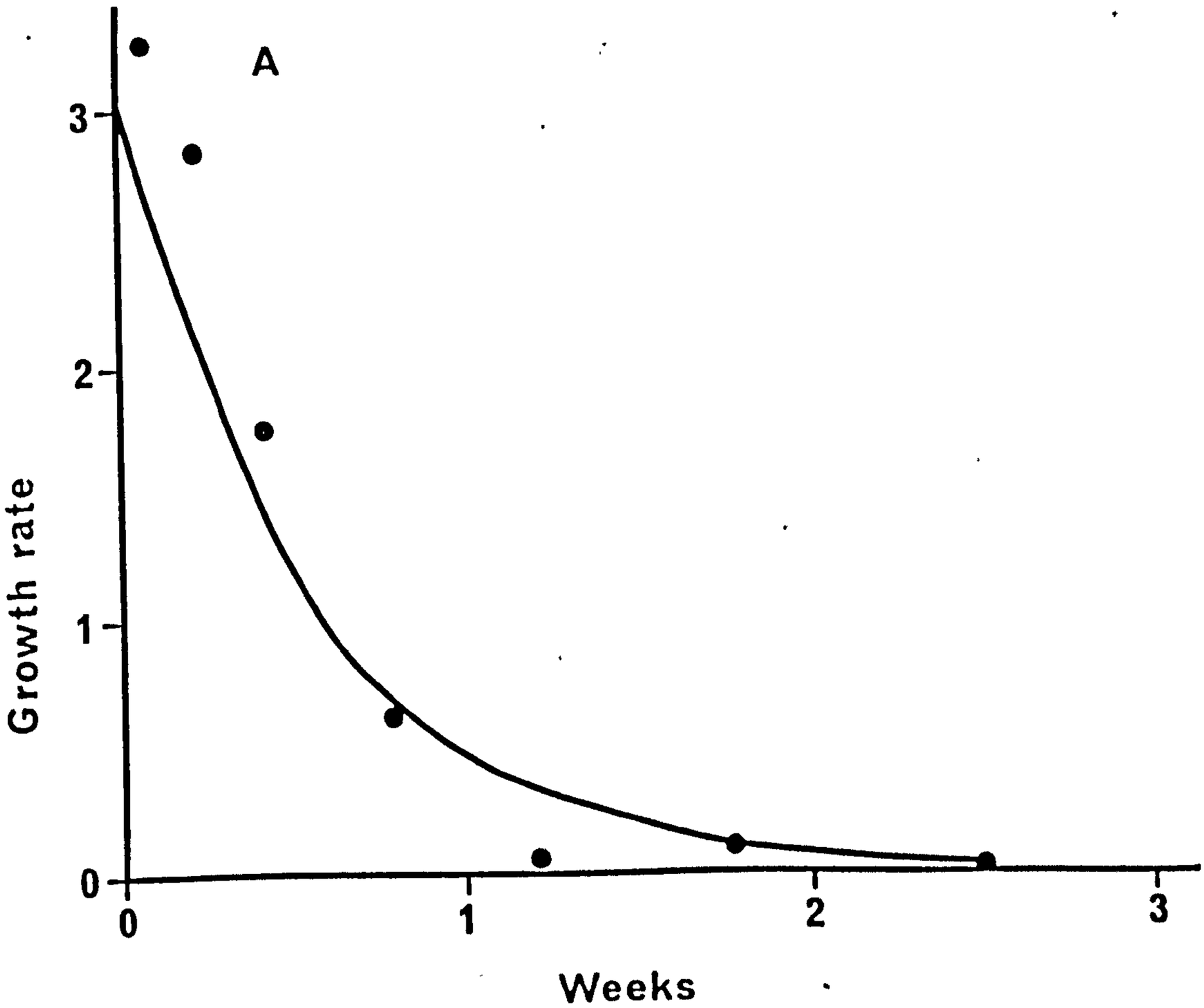
1. The solid circles denote the observed points.
2. The solid line is the curve predicted by an exponential model (equation 31).

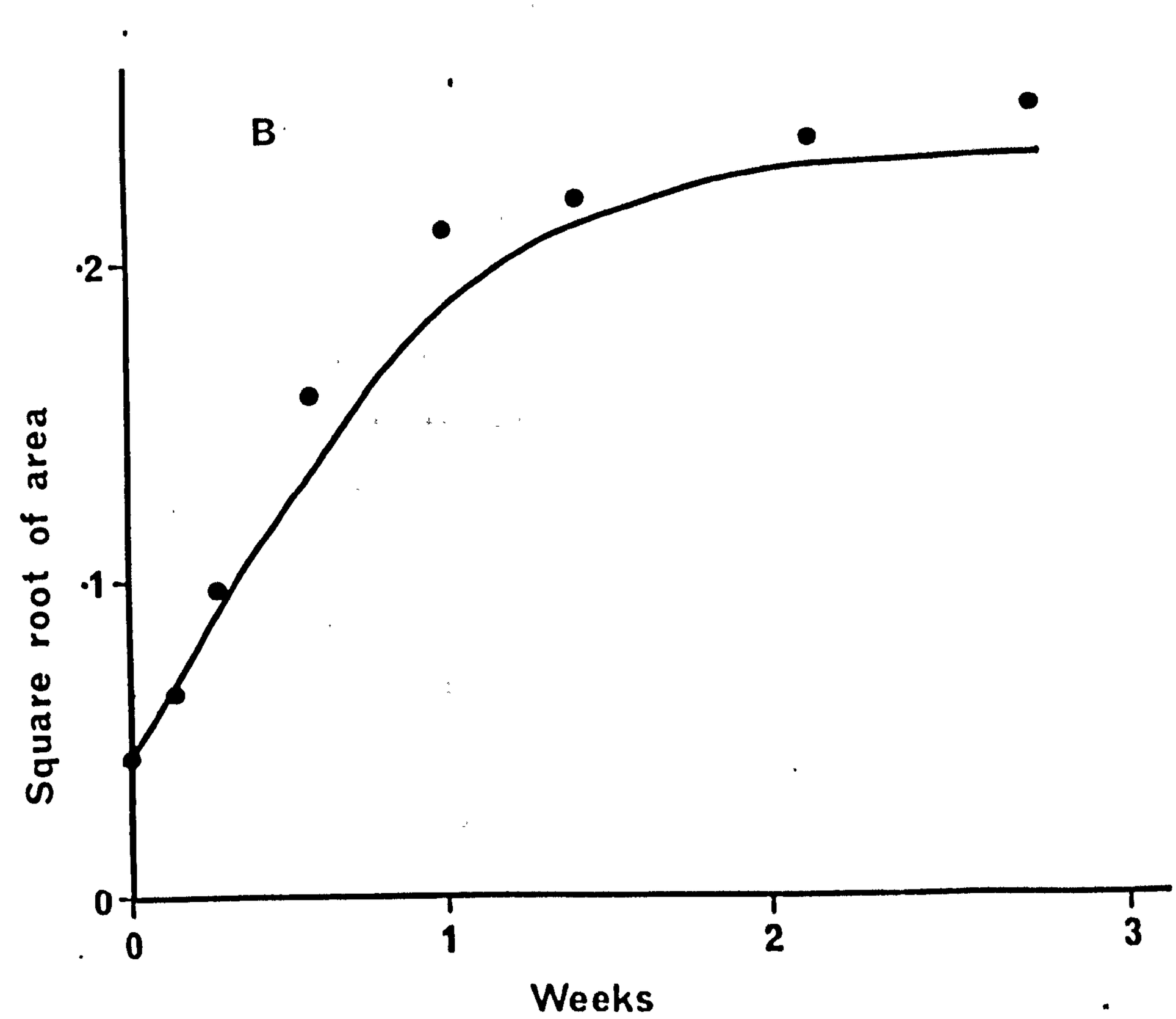
For the values of the coefficients for the model see table 40C.

Fig. 54

The square root of the mean area of the vitelline glands of T.patialense at a series of consecutive points in time post infection.

1. The solid circles denote the observed points.
2. The point at t=0 is the value at the time of infection and is the result for decaudated cercariae.
3. The solid line is the curve predicted by a Gompertz growth model (equation 28) with the same coefficients as in fig.53 above.


A
3
2
1
0
Growth rate
0
1
2
3
Weeks


B
·2
·1
0
Square root of area
0
1
2
3
Weeks

of the growth in area in fig. 54 (table 41D). Fig55 (table 41C) shows the observed data for the area of the vitelline glands as a percentage of the total area of the flukes. This graph shows that almost all the expansion of the vitelline area as a percentage of the total area occurs in the first week post infection. Most of the increase in area of the vitelline glands after this time is accompanied by an equivalent increase in total area.

b) Assessment of reproductive abnormalities in adult T.patialense.

Figure 56 and table 42A show the mean proportion of flukes showing reproductive abnormalities following initial parasite densities of 14 flukes per host at 23°C on successive weeks post infection. The proportion of abnormal flukes is low except in weeks six, seven and eight where between 16.3 and 22.5% were affected. The majority of the abnormalities consisted of the presence of between one and five tanned eggs in the uterus with or without a large amorphous mass of tanned vitelline material in the vitelline reservoir and ootype. Less often such a mass of material was present in the absence of tanned eggs. Plate 5 shows a typical "blocked fluke" with three tanned eggs in the uterus.

When tanned eggs were seen in the uterus of a fluke in the one fluke per host density dependent fecundity and survival experiments, egg production was assessed over five successive 24 hour periods. On every one of these occasions no eggs were produced. From this result the assumption was made that there was no egg production in any fluke showing these abnormalities. An attempt was made to investigate the influences of these abnormalities on the rate of egg production per surviving fluke.

The mean rate of egg production per surviving fluke at an initial parasite density of 14 flukes per host at 23°C (fig. 57, table 42B) was re-determined on the basis of only those flukes not

PLATE 5.

Plate 5

An adult fluke displaying reproductive abnormalities.

A. A series of three tanned eggs are present in the uterus.

B. An amorphous mass of tanned vitelline material is present in the ootype.

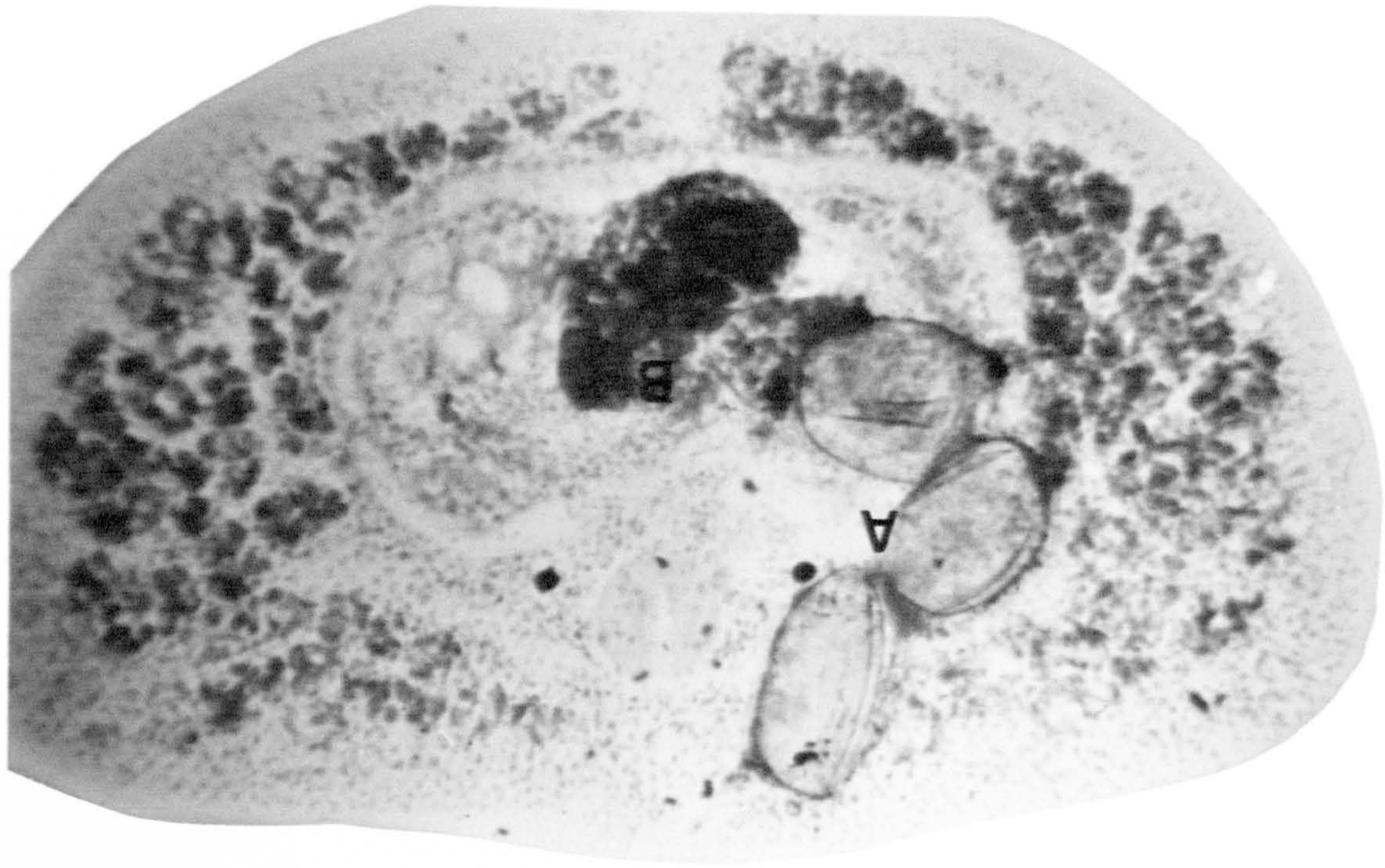
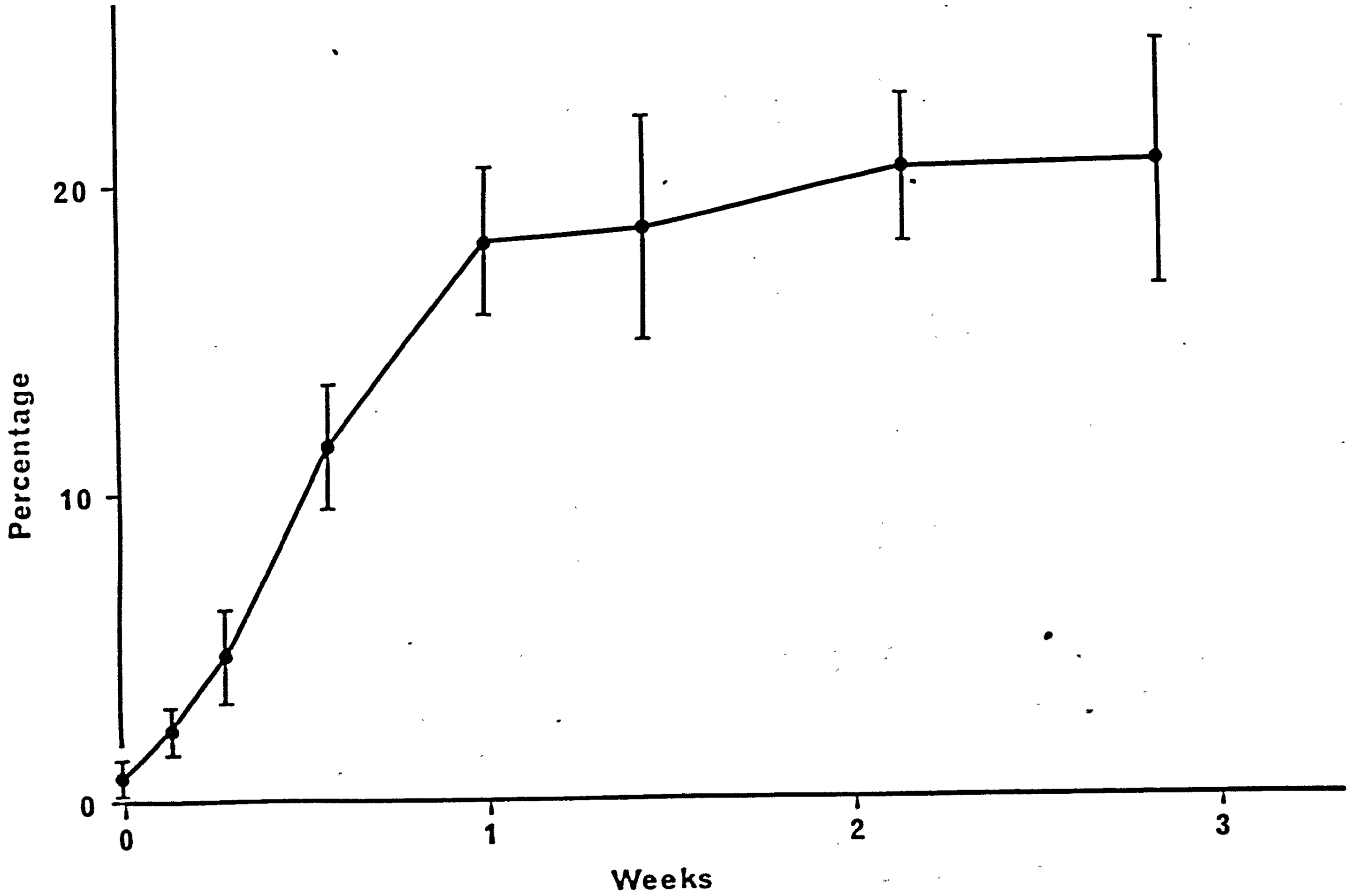

B
A

FIG.55.

Fig. 55

The area of the vitelline glands of T.patialense as a percentage of total area at a series of consecutive points in time post infection.

1. The solid circles denote the means of sets of observed points.
2. The point at t=0 is the value at the time of infection and is the result for decaudated cercariae.
3. The vertical bars denote the 95% confidence limits round the points.


20
10
0
Percentage
0
1
2
3
Weeks

displaying reproductive abnormalities as predicted by the data in table 42A. The resulting data for egg production per surviving normal fluke are shown in table 42C and the increases over egg production for all surviving flukes in fig. 57. It is clear that the assumption that abnormal flukes exhibit no egg production has only a small effect on the previously calculated rate of egg production. Thus the increase in the proportion of abnormal flukes at six, seven and eight weeks post infection only goes a small way towards explaining the falling phase of egg production per surviving fluke which commences after the third and fourth weeks post infection at 23°C.

c) Feeding.

To investigate the mode of feeding of T.patialense on its experimental definitive host B.rerio scanning electron micrographs of the surface of infected fish were produced. When scales, under which parasites were present, were removed, impressions made in the underlying epidermis by the ventral sucker were visible at low power. Plate 6 shows one such impression.

At higher magnifications a lesion (plate 7, C) was observed at the anterior side of the ventral sucker impression (plate 7, B) with respect to the anterior posterior axis of the fish host. This portion of the impression is consistent with the area of overlap between the ventral sucker and oral sucker or pharynx of the parasite (fig. 3). It thus appears that this lesion represents feeding damage by the parasite to its fish host.

FIGS.56,57.

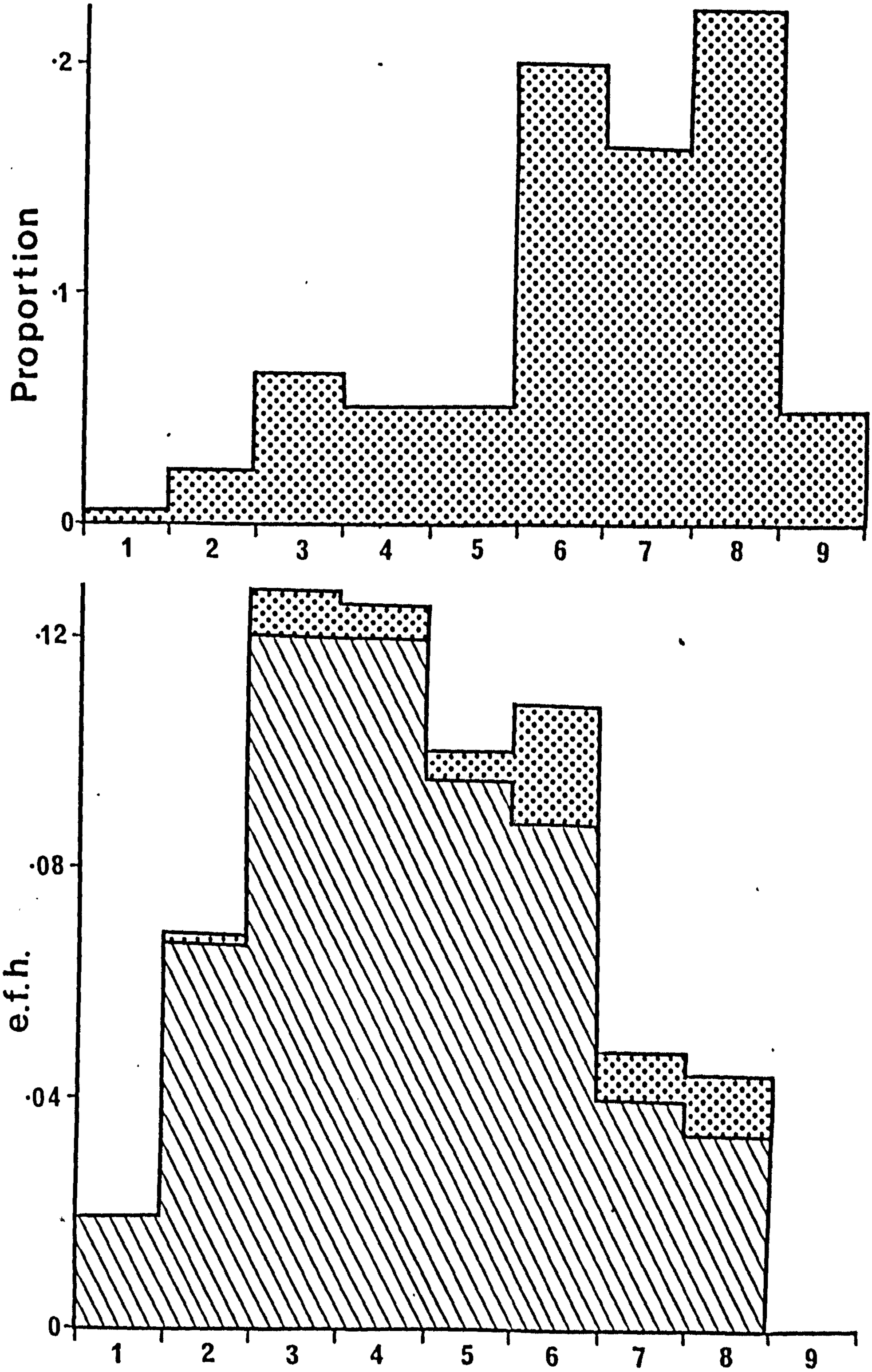
Fig. 56

The mean proportion of surviving flukes showing reproductive abnormalities in successive weeks post infection.

Fig. 57

The mean rate of egg production per fluke per hour in successive weeks post infection with an initial parasite density of 14 flukes per host at 23°C.

1. The hatched areas show the mean rate for all surviving flukes.
2. The stippled areas show the increase if the rate is re-calculated on the basis of flukes not showing reproductive abnormalities.


Proportion
·2
·1
0
1
2
3
4
5
6
7
8
9
e.f.h.
·12
·08
·04
0
1
2
3
4
5
6
7
8
9
Weeks

PLATES 6,7.

Plate 6

Scanning electron micrograph of a portion of the surface of a specimen of Brachydanio rerio infected with Transversotrema patialense.

Parts of a number of scales are visible but in the central area a scale beneath which a specimen of T.patialense was present has been removed. The imprint of the parasites ventral sucker can be seen in this area (A).

The top of the picture is anterior with respect to the anterior-posterior axis of the fish.

Plate 7

Scanning electron micrograph showing the imprint of the ventral sucker of Transversotrema patialense on Brachydanio rerio in greater detail.

A. The indentations made by the backwardly directed spines covering T.patialense.

B. The imprint of the ventral sucker in greater detail.

C. Imprint of mouth. The picture is rotated 90 degrees anti-clockwise from Plate 6 and so the depression shown is in the position where the oral sucker, or pharynx, of T. patialense overlaps the ventral sucker and is presumed to be a feeding lesion.

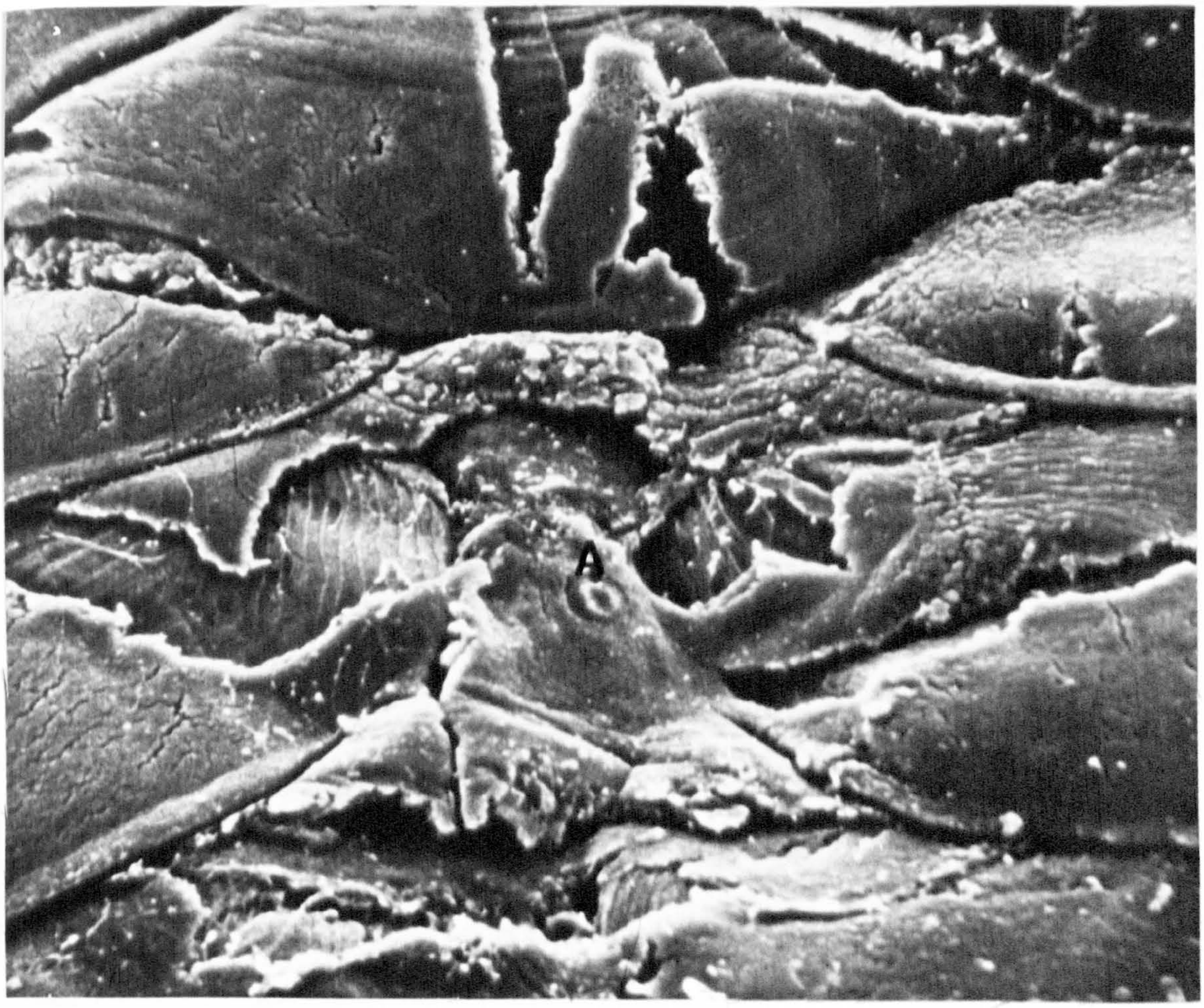

A
·5mm
B
C
A
·05mm

Table 38 Instantaneous growth rates for the widths of adult T.patialense at three different initial densities per host at a series of consecutive points in time. Observed values and values predicted by an exponential model (equation 31).

	14 flukes per host		30 flukes per host		124 flukes per host	
	Instantaneous growth rate		Instantaneous growth rate		Instantaneous growth rate	
Time (weeks)	observed	calculated	observed	calculated	observed	calulated
.5	.265	.311	.237	.271	.195	.195
1.5	.121	.113	.123	.123	.093	.093
2.5	.059	.040	.100	.061		
3.5	.011	.014	.021	.029		
a (intercept)		.261		.395		.282
b (slope)		-1.026		- .7478		- .743
r		.930		.939		-

Table 39 Mean widths of adult T.patialense at three different initial densities per host at a series of consecutive points in time. Observed values with 95% confidence limits and values predicted by growth model (equation 28).

Time (weeks)	14 flukes per host			30 flukes per host			124 flukes per host		
	observed mean width (mm)	95% confidence limits	width predicted by growth model	observed mean width (mm)	95% confidence limits	width predicted by growth model	observed mean width (mm)	95% confidence limits	width predicted by growth model
0	.638	.006	.638	.638	.006	.638	.638	.006	.638
1	.832	.012	.886	.809	.013	.842	.763	.020	.778
2	.939	.019	1.000	.915	.018	.961	.837	.032	.856
3	.996	.018	1.040	1.011	.024	1.027			
4	1.007	.021	1.056	1.032	.022	1.053			

Table 40 Observed values and values predicted by the exponential growth model for instantaneous growth rates.

	Area of flukes		Area of vitellaria		$\sqrt{}$ of area of vitellaria	
	Instantaneous growth rate		Instantaneous growth rate		Instantaneous growth rate	
Time (weeks)	observed	calculated	observed	calculated	observed	calculated
.071	.7629	.7166	6.580	5.444	3.287	2.741
.214	.7237	.6131	5.802	4.158	2.908	2.090
.429	.4780	.4848	3.650	2.772	1.815	1.390
.786	.2669	.3284	1.278	1.415	.645	.707
1.214	.1825	.2058	.166	.631	.085	.314
1.786	.1114	.1102	.248	.215	.120	.106
2.500	.0560	.0506	.092	.056	.045	.022
a (intercept)		.7744		6.224		3.136
b (slope)		-1.0916		-1.885		-1.896
r (correlation)		.971		.930		.941

Table 41 Observed growth data with 95% confidence limits and values predicted by the growth model at a series of consecutive points in time.

	A. Area of flukes			B. Area of vitellaria			C. Vitellaria as % of total	
Time (weeks)	observed mean area (mm^2)	95% confidence limits	area calculated using growth model	observed mean area (mm^2)	95% confidence limits	area calculated using growth model	observed percentage	95% confidence limits
0	.158	.013	.158	.002	.001	.002	.819	.60
.143	.176	.014	.175	.004	.002	.003	2.33	.87
.286	.195	.021	.190	.009	.004	.006	4.81	1.65
.571	.220	.012	.220	.027	.006	.014	11.65	2.13
1.000	.247	.016	.253	.046	.008	.026	18.23	2.44
1.429	.267	.020	.277	.050	.009	.035	18.58	3.62
2.143	.289	.015	.300	.059	.006	.041	20.48	2.39
2.857	.301	.021	.311	.063	.014	.043	20.70	3.95

cont...

Table 41 continued

Time (weeks)	D. Square roots of area of vitellaria	
	observed mean square root	value predicted by growth model
0	.045	.045
.143	.064	.067
.286	.097	.090
.571	.163	.134
1.000	.215	.184
1.429	.223	.211
2.143	.243	.229
2.857	.251	.234

Table 42

	A	B	C
Time (weeks)	Mean proportion of surviving flukes showing reproductive abnormalities, 14 flukes per host, 23°C.	Mean egg production per surviving fluke per hour (e.f.h.), 14 flukes per host, 23°C.	Mean egg production per hour per surviving fluke not displaying reproductive abnormalities, 14 flukes per host, 23°C.
0-1	.0059	.019	.019
1-2	.0175	.067	.068
2-3	.0659	.120	.128
3-4	.0488	.120	.126
4-5	.0490	.095	.100
5-6	.200	.087	.108
6-7	.163	.040	.048
7-8	.225	.034	.044
8-9	.050	-	-

CHAPTER 9

The effect of cyclical changes in illumination and temperature on egg production.

During trials to develop an accurate method to assess fecundity, it was noted that egg production appeared to be higher during periods of darkness than during periods of light.

This interesting phenomenon was investigated in more detail. In each experiment a newly infected host was placed in a cage and rotated automatically through a series of pots of tapwater using a modified laboratory carousel. Eggs from the parasites on the fish fell into the water in the dishes and were counted. The effects of cyclical changes in temperature and illumination on the rate of egg production were investigated.

It was found that in all the experiments the first eggs were produced between 72 and 96 hours post infection. Egg output was consistently greater during periods of darkness than during periods of illumination in both replicates (fig. 58A; table 43). These differences were found to be significant using a modified version of Student's t-test for small samples (Bailey, 1959) to test total egg production in each light period against total egg production in each dark period (table 43).

$t = 5.012$ 10 df $P < .001$

$t = 7.699$ 20 df $P < .001$

In the control experiments conducted in conditions of constant light, egg production in the 12 hour periods corresponding temporarily to the periods of light and dark, showed no obvious rhythm (fig. 59C, D; table 43). The difference between the periods was found to be statisically insignificant in each case.

$t = .943$ 18 df $P > 0.10$

$t = .074$ 22 df $P > 0.10$

FIG.58.

Fig. 58

Egg production of T.patialense in cyclically varying light (A and B), constant light (C and D) and temperature (E) regimes, expressed as a proportion of the highest recorded production in a 12 hour period in each experiment.

1. In A-D the diagonal lines represent light periods and the heavily outlined bars, dark periods.
2. In E the diagonal lines represent periods at 26°C and the dotted areas represent periods at 23°C.

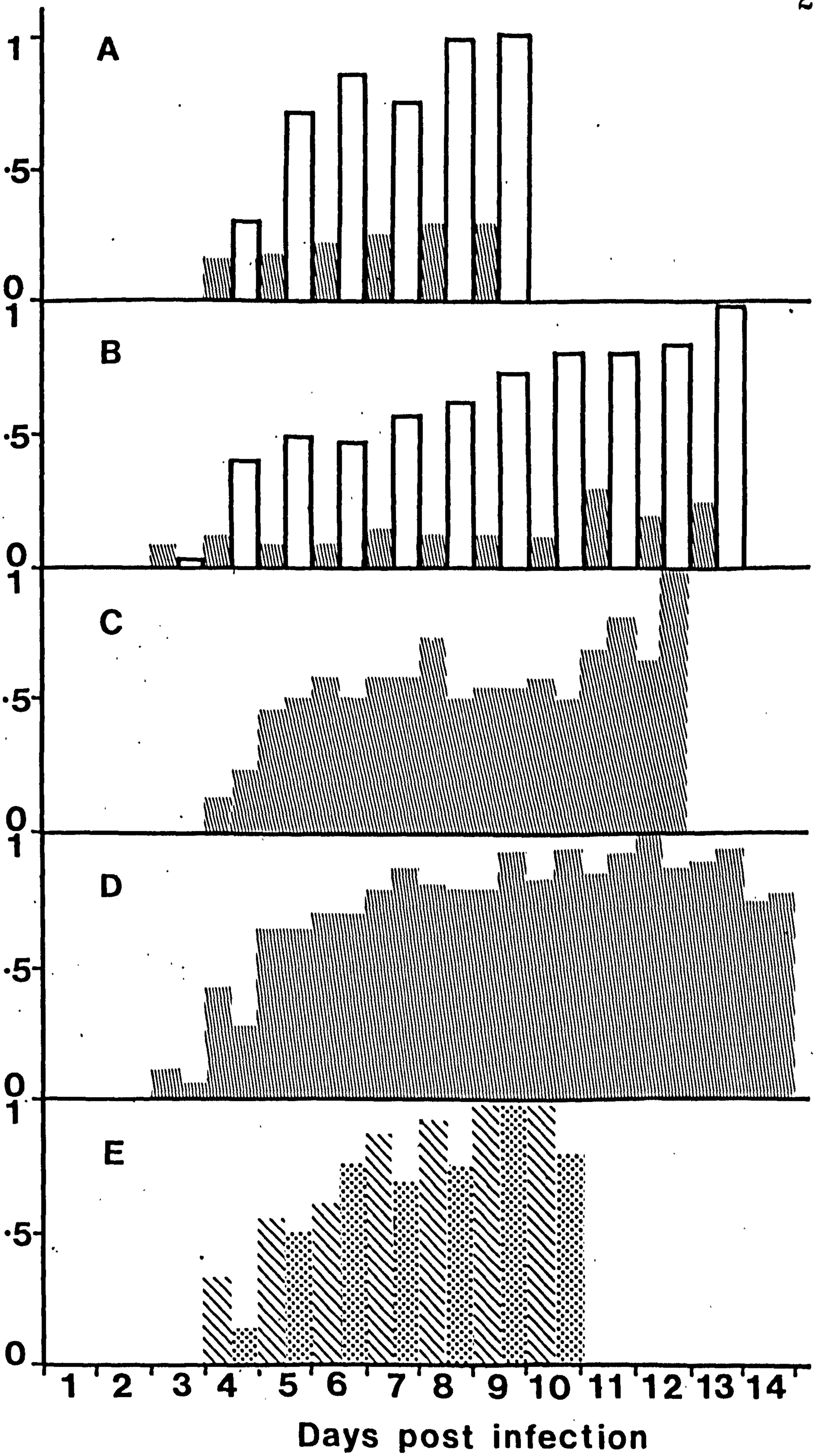

A
B
C
D
E
1
·5
0
Egg production
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post infection

FIG.59.

Fig. 59

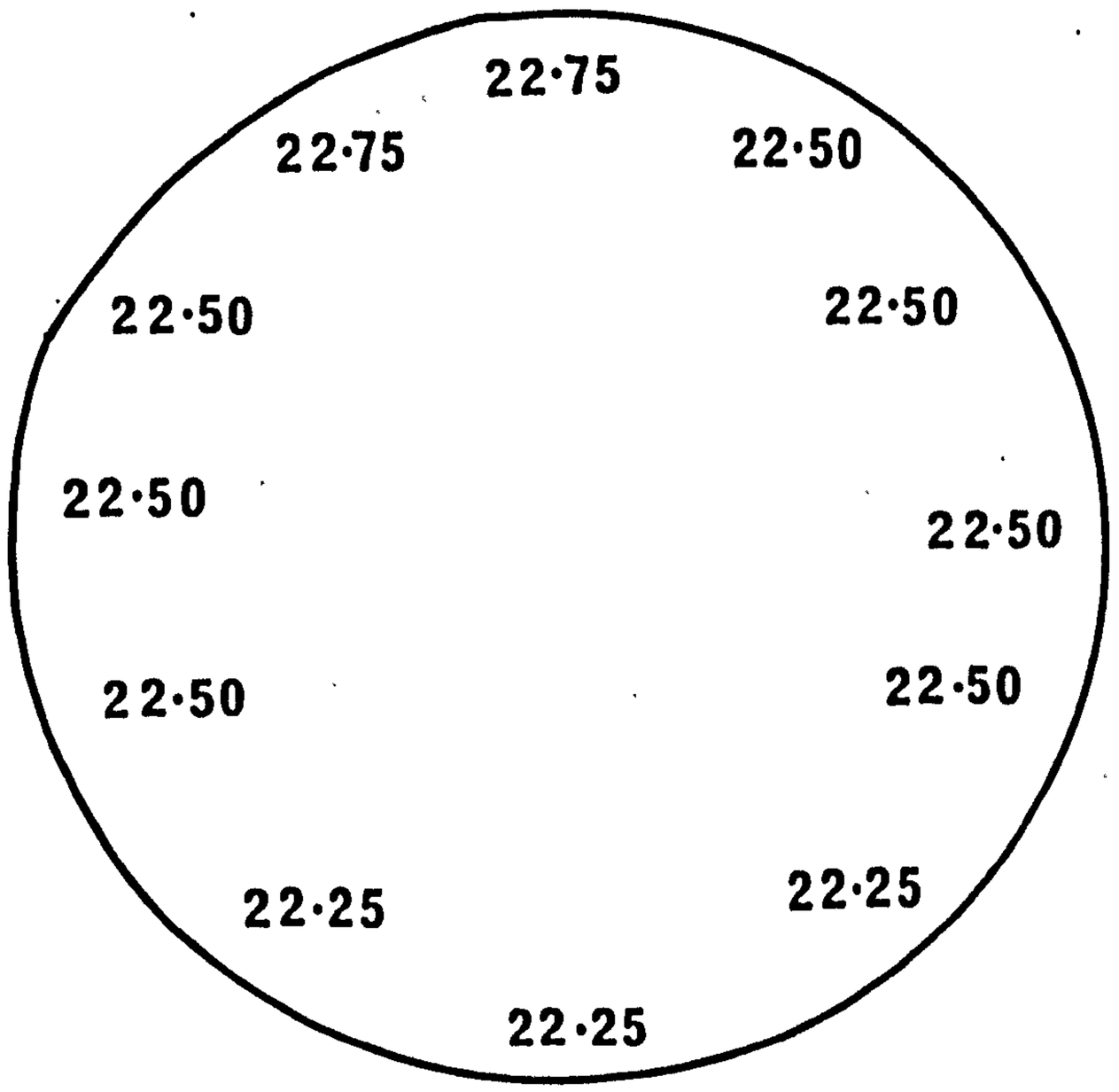
Temperature changes in egg collecting pots due to illumination.

A. Temperatures in pots after six hours of darkness.

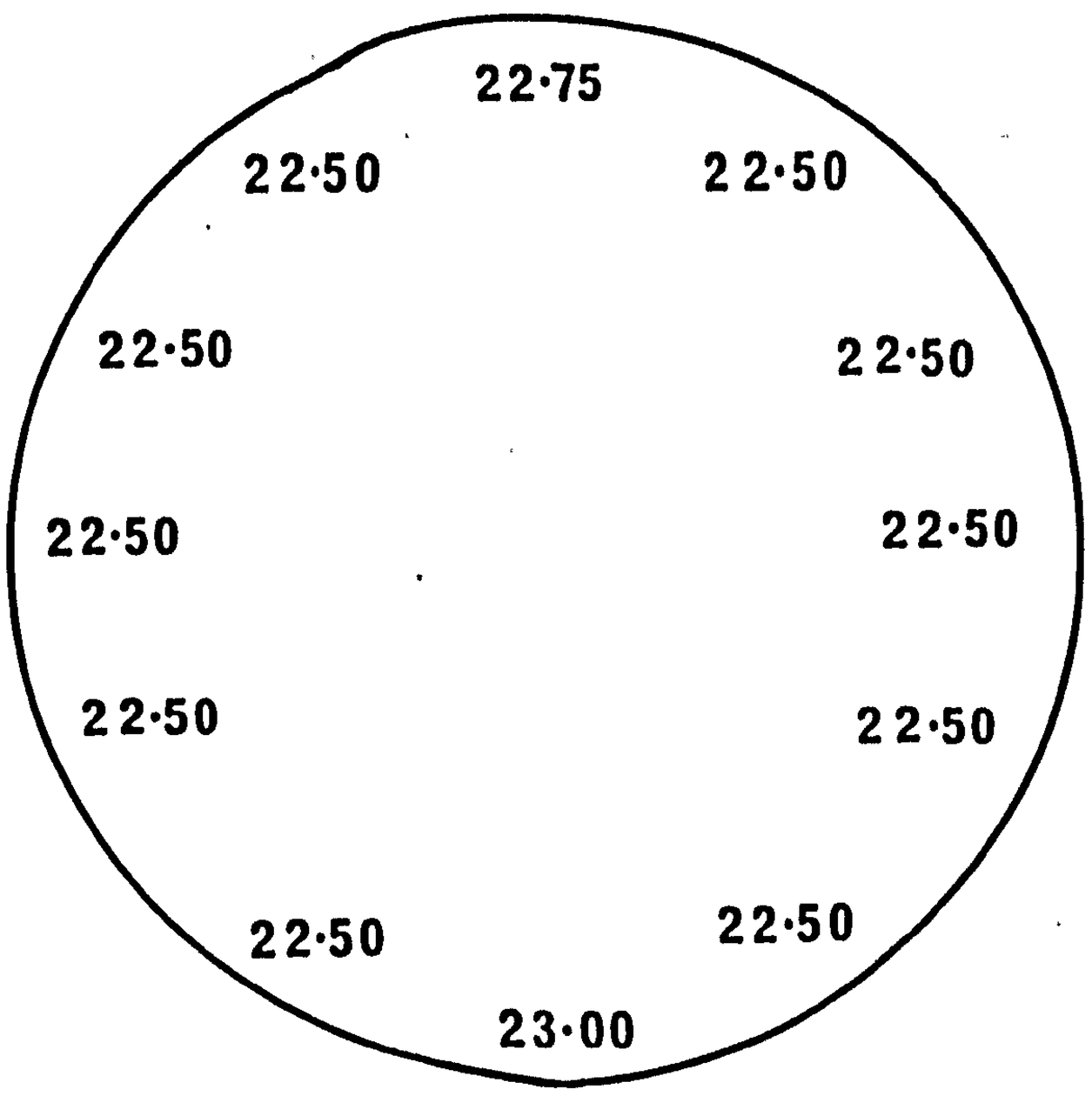
B. Temperatures in pots after four hours illumination.

a. Light source.

A


22·75
22·75
22·50
22·50
22·50
22·50
22·50
22·50
22·25
22·25
22·25

B


22·75
22·50
22·50
22·50
22·50
22·50
22·50
22·50
22·50
22·50
23·00
a

In the experiment using a cyclically varying temperature regime, egg production tended to be slightly greater during the 12 hour periods at 26°C than during the 12 hour periods at 23°C (fig. 57 E; table 43). This difference was found to be statistically insignificant

$t = 1.746$ 14 df $P > 0.1$

In the control experiment to determine the heating effects of the fluorscent tube used to provide illumination, the pot of tapwater nearest the light rose in temperature by 0.75°C over a four hour period. The temperatures in the other pots varied by no more than $\pm$ 0.25°C (fig. 58), the temperature of the experimental chamber being 23°C.

So the temperature differences in the pots were extremely small, and the temperature dependent factors in the rate of egg production are simlarly small. Thus, the observed differences in the cyclically varying illumination regime, appear to be a direct result of the actual illumination changes, and are not due to the heating effects of the light source.

Table 43 Egg production per host during twelve hour periods.

Days post inf.	Cyclical changes in illumination		Cyclical changes in illumination		Constant illumination	Constant illumination	Constant illumination	Constant illumination	Cyclical changes in temperature	
	light	dark	light	dark	light	light	light	light	26°C	23°C
1										
2										
3			6	2			5	3		
4	9	17	9	30	4	6	20	13	12	5
5	10	39	7	37	12	16	30	30	20	18
6	12	47	7	35	15	16	33	33	22	27
7	14	41	10	43	15	15	37	41	31	19
8	16	54	9	46	19	13	38	37	31	25
9	16	55	9	55	14	14	44	39	33	27
10			8	61	15	16	44	40	36	35
11			22	60	18	21	43	40	36	28
12			14	62	17	26	41	42		
13			18	75			45	35		
14							36	34		
Mean	12.84	42.18	10.80	46.00	14.34	15.9	34.67	32.25	27.63	23.00

CHAPTER 10

The influence of differing ionic environments on the cercarial, post cercarial and adult stages of Transversotrema patialense.

The survival of adult T.patialense and survival and infectivity of cercariae in differing ionic environments were investigated. These experiments constituted an attempt to gain insights into the ionic environment of the adult fluke in its unusual niche under the scales of its fish host. It was also hoped that the results of such experiments would enable a comparison between the transformation from cercaria to post-cercarial and adult stages with the similar transformation of the entoparasitic digenean Schistosoma mansoni which has been extensivley studied (Brink, McLaren and Smithers, 1977; Ramalho-Pinto, Gazzinelli, de'Oliveira, Figueiredo and Pellegrino, 1975). Lastly, it was believed that investigations using a variety of ionic environments would provide information relevant to the existance of marine, and brackish, as well as freshwater, definitive hosts for the genus Transversotrema (Appendix 1).

The egg output of adult flukes in vitro was also investigated to determine whether there were any illumination-generated differences in egg output. The existance of such differences might indicate that the cyclical egg output of the parasites (chapter 9) is due to the direct effects of light on the occelate adult parasite, rather than through light generated rhythms in host behaviour or physiology.

a) The in vitro culture of adult T.patialense.

a1) The influence of different saline solutions on the survival of adult flukes in vitro.

Adult flukes were removed from the fish host and their survival investigated in different saline solutions. The survival

curves for adult flukes removed from their fish hosts three days post infection, and cultured in full strength Cortland saline, in Cortland saline made up with tapwater instead of distilled water, and in frog ringer, are all extremely similar (fig. 60, table 44A). It appears from these results that there was no factor in tapwater reducing survival and that the glucose (1gm/litre) in the Cortland saline did not increase survival appreciably over the frog ringer which did not contain glucose.

The survival curves for adult flukes removed from their fish hosts eleven days post infection (fig. 61, table 44B) show that, whilst a reduction in the strength of Cortland saline to 50% causes a small decrease in survival, a reduction to 10% results in a steep initial decline. Only half the original number remain after 45 minutes in the saline. After this steep fall the decline in the proportion surviving becomes progressively more gentle and after 50 hours the proportions surviving in 10% and 50% saline are similar.

a2) <u>The effect of age at the time of removal from the fish host on the survival of adult flukes</u>.

i) <u>Full strength Cortland saline</u>.

From fig. 62 it is clear that the survival curves for adult flukes removed from the fish host three and eleven days post infection are very similar. The curve for flukes removed from the host five minutes post infection is rather lower. From the sets of observed survival data (table 45) the instantaneous death rates were determined at a series of consecutive points in time (table 46). Least squares linear regressions were fitted to the natural logarithms of the three sets of data. For all three regressions the correlation coefficients were highly significant ($P < .01$). The analysis of variance described in chapter 6 was used to determine whether the regressions for the transformed death rates of five minutes, and

FIGS.60,61.

Fig. 60

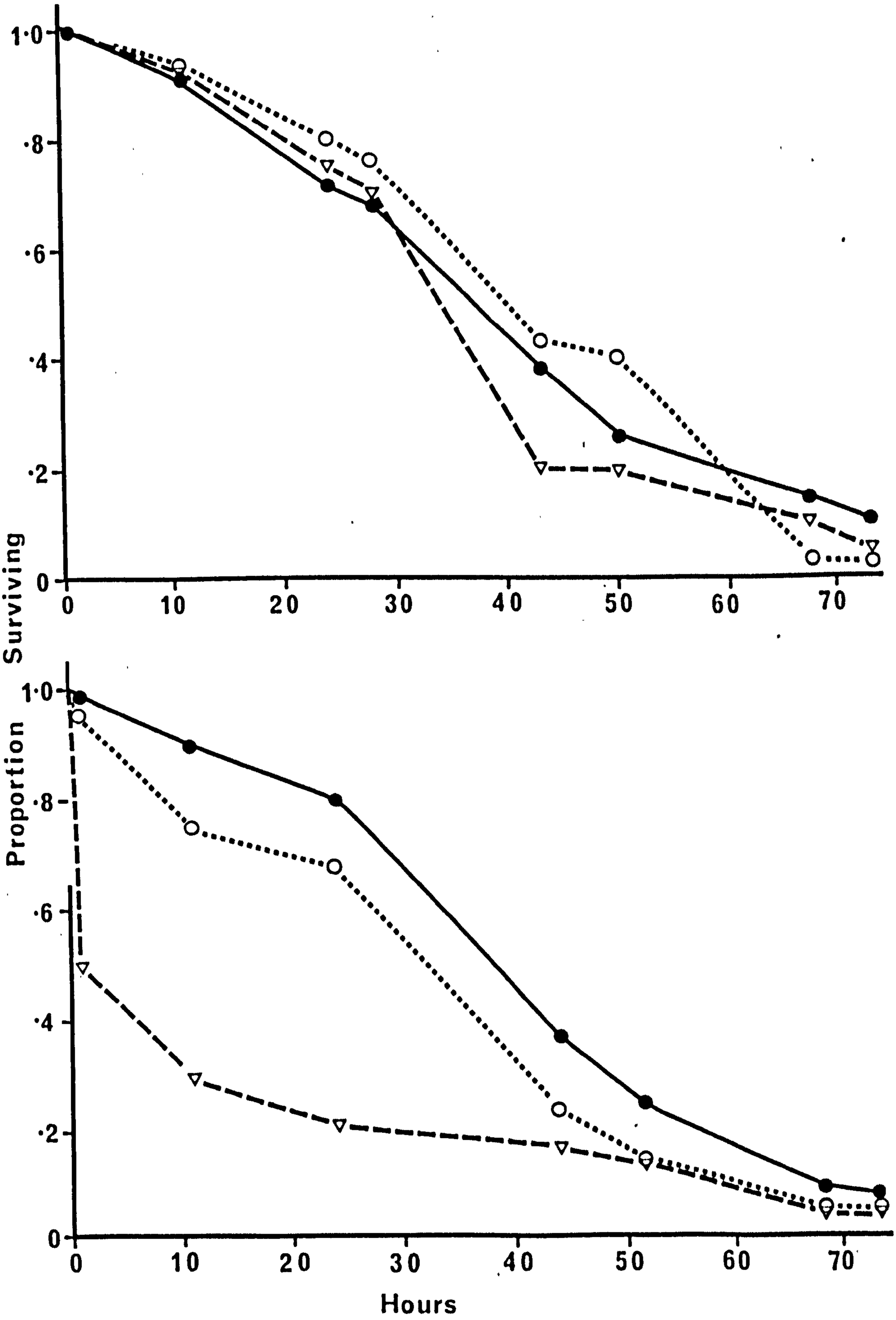
The proportion of flukes removed from the fish host three days post infection surviving at a series of consecutive points in time in different culture media.

1. Solid circles denote survival in full strength Cortland saline.
2. Open circles denote survival in full strength Cortland saline made up with tapwater instead of distilled water.
3. Open triangles denote survival in full strength frog ringer.

Fig. 61

The proportion of flukes removed from the fish host eleven days post infection at a series of consecutive points in time in different culture media.

1. Solid circles denote survival in full strength Cortland saline.
2. Open circles denote survival in 50% Cortland saline.
3. Open triangles denote survival in 10% Cortland saline.


1·0
·8
·6
·4
·2
0
0
10
20
30
40
50
60
70
Proportion Surviving
1·0
·8
·6
·4
·2
0
0
10
20
30
40
50
60
70
Hours

FIGS.62,63.

Fig. 62

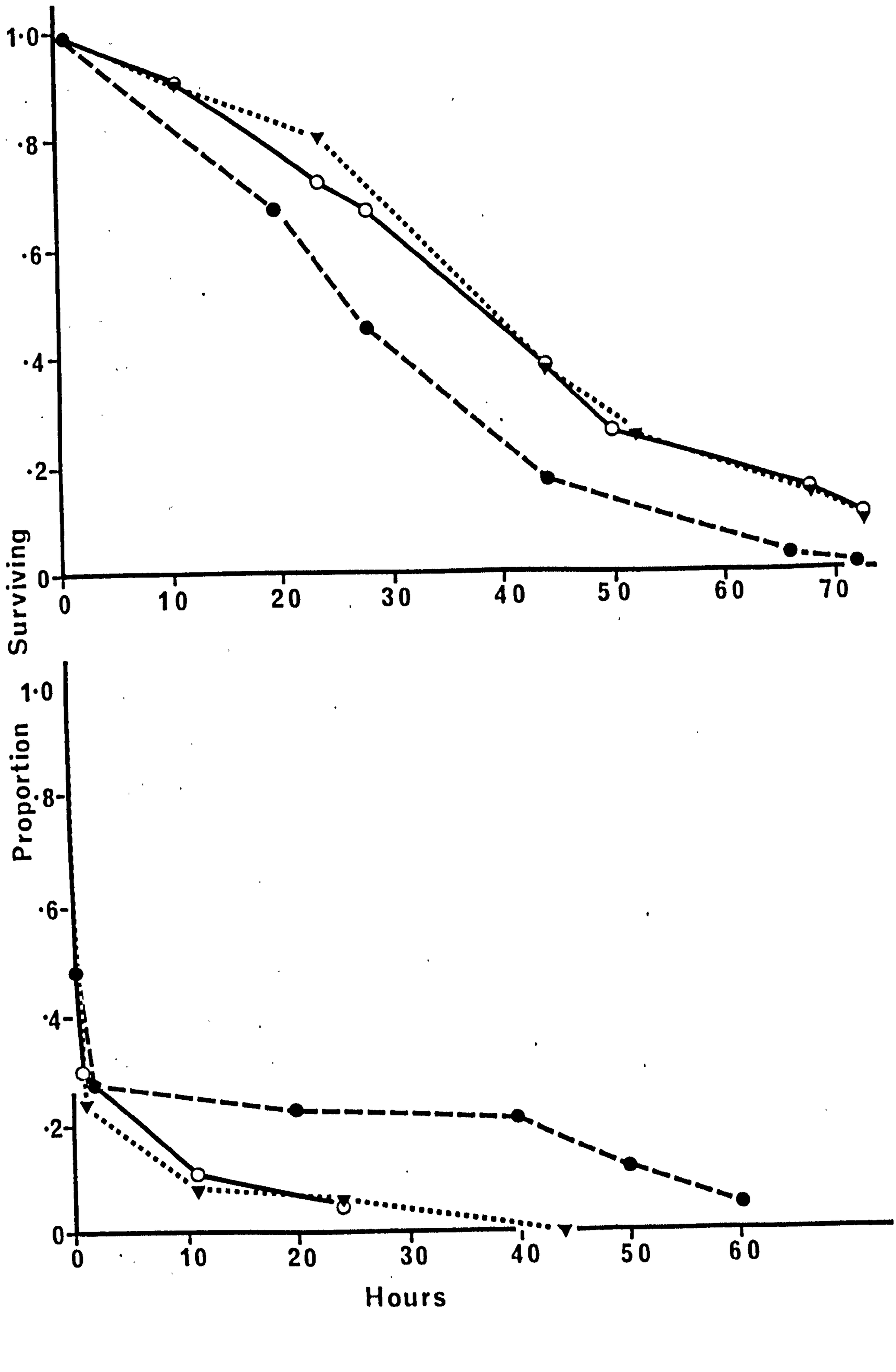
The proportion of flukes surviving at a series of consecutive points in time in full strength Cortland saline.

1. Solid triangles denote flukes removed from the fish host eleven days post infection.
2. Open circles denote flukes removed from the fish host three days post infection.
3. Solid circles denote flukes removed from the fish host five minutes post infection.

Fig. 63

The proportion of flukes surviving at a series of consecutive points in time in tapwater.

1. Solid triangles denote flukes removed from the fish host eleven days post infection.
2. Open circles denote flukes removed from the fish host three days post infection.
3. Solid circles denote flukes removed from the fish host five minutes post infection.


1·0
·8
·6
·4
·2
0
0
10
20
30
40
50
60
70
Surviving
Proportion
1·0
·8
·6
·4
·2
0
0
10
20
30
40
50
60
Hours

three day, post infection flukes (fig. 64) and three and eleven day, post infection flukes (fig. 65), were significantly different.

It was found that there was no significant difference between either the slope or the intercept of the regressions for the three and eleven day post infection flukes ($P > .10$). There was no significant difference between the slopes of the regressions for five minute and three day flukes ($P > .10$), but there was a significant difference between the intercepts ($P < .01$). These results support the deductions made from the survival curves that flukes removed from the fish host after five minutes post infection survive less well in the Cortland saline than those removed three and eleven days post infection.

ii) Tapwater

In tapwater the proportion of flukes surviving falls steeply to between .278 and .238 of the original level after one to two hours, for all three ages, post infection. After this time the proportion of flukes removed three and eleven days post infection are similar, and fall to .05 and .062 of the original level 24 hours post removal. The proportion of flukes removed five minutes post infection falls much less steeply, and .226 survive for 20 hours and .212 for 40 hours *in vitro*.

Looking at figures 62 and 63 together, flukes removed five minutes post infection survive less well in saline, and better in tapwater, than flukes removed from the fish host three and eleven days post infection.

a3) Survival of flukes in vitro in full strength Cortland saline in non-sterile conditions and in full strength Hank's solution in sterile conditions.

From figs. 66 and 67 it appears that flukes survived rather less well in sterile Hank's solution than in non-sterile Cortland

FIGS.64,65.

Fig. 64

Natural logarithmic transformations of the instantaneous death rates (equation 1) of flukes <u>in vitro</u> at a series of consecutive points in time in full strength Cortland saline.

1. The open circles show the observed data for flukes removed from the fish host three days post infection and the dashed line is a regression fitted to this data with the coefficients:

   a (intercept) = -4.7622

   b (slope) = .03177

2. The solid circles show the observed data for flukes removed from the fish host five minutes post infection and the solid line is a regression fitted to this data with the coefficients:

   a (intercept) = -4.2036

   b (slope) = .0333

Fig. 65

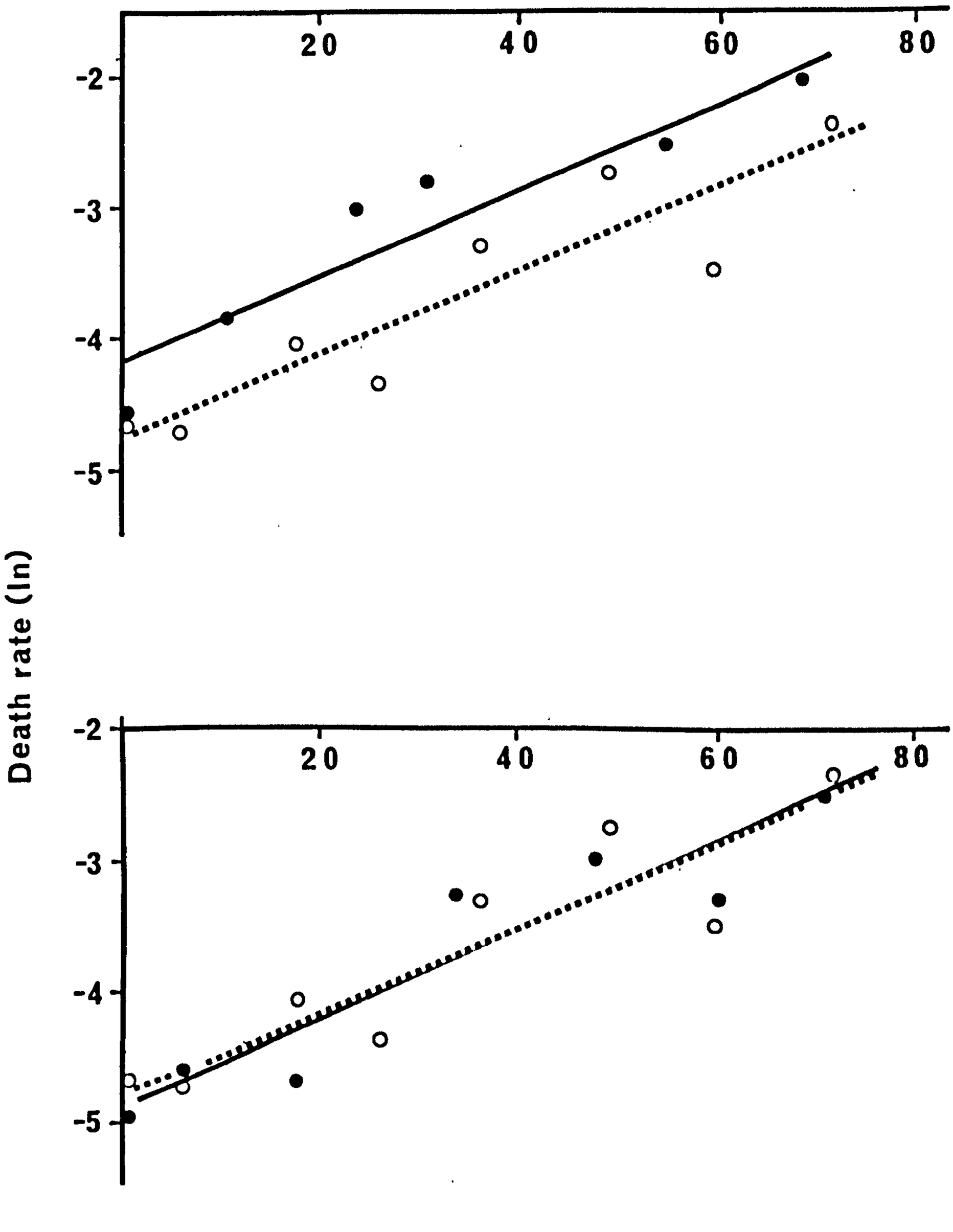
Natural logarithmic transformations of the instantaneous death rates of flukes <u>in vitro</u> at a series of consecutive points in time in full strength Cortland saline.

1. The solid circles show the observed data for flukes removed from the fish host eleven days post infection and the solid line is a regression fitted to this data with the coefficients:

   a (intercept) = -4.938

   b (slope) = .0355

2. The open circles and dashed line are identical with 1. in fig. 64.


20
40
60
80
-2
-3
-4
-5
Death rate (ln)
Hours

FIGS.66,67.

Fig. 66

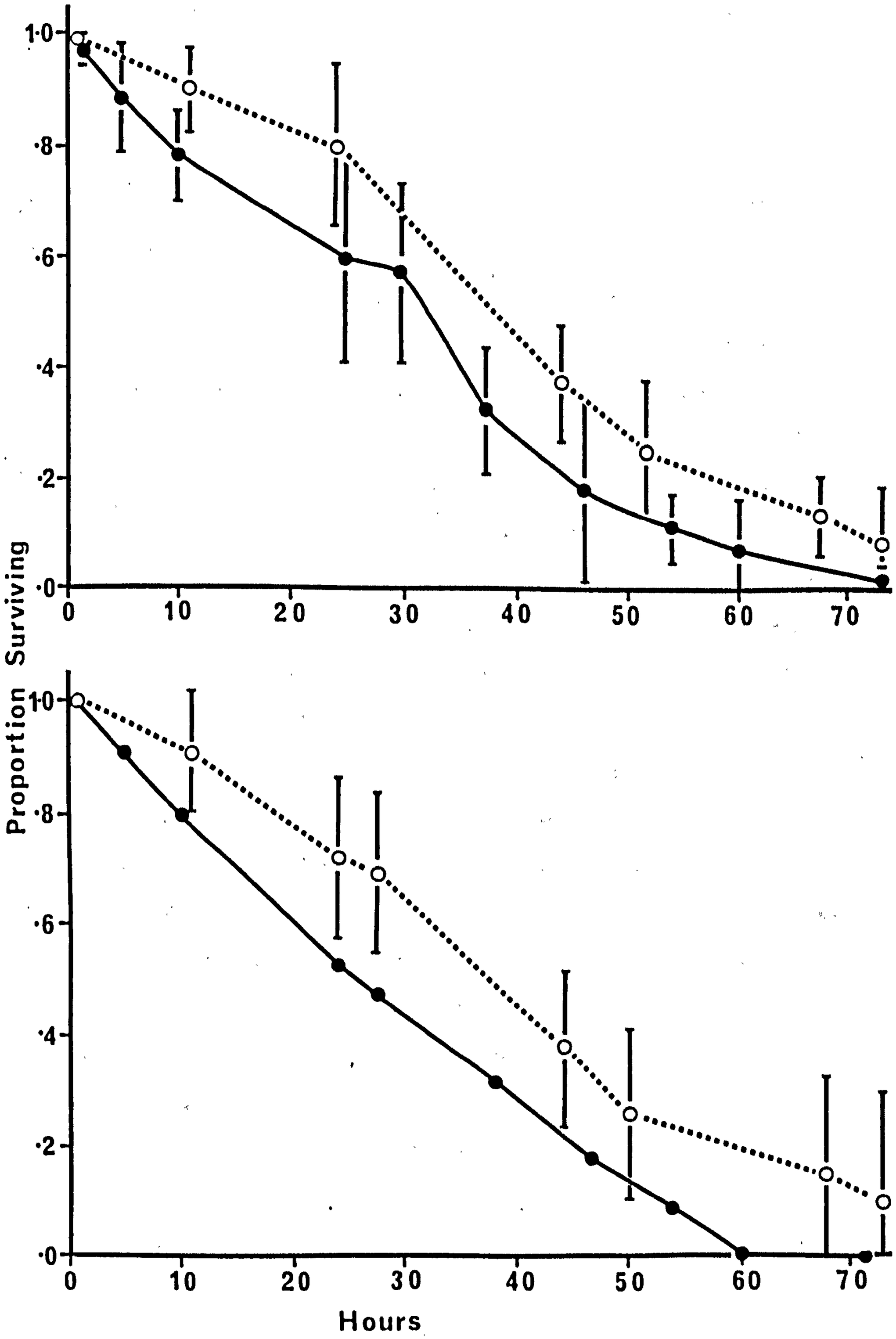
The proportion of flukes removed from the fish host eleven days post infection, surviving <u>in vitro</u> at a series of consecutive points in time.

1. Open circles denote survival in full strength Cortland saline in non-sterile conditions.
2. Solid circles denote survival in full strength Hank's solution in sterile conditions.
3. The vertical lines denote 95% confidence limits.

Fig. 67

The proportion of flukes removed from the fish host three days post infection surviving <u>in vitro</u> at a series of consecutive points in time.

1. Open circles denote survival in full strength Cortland saline in non-sterile conditions.
2. Solid circles denote survival in full strength Hank's solution in sterile conditions.
3. The vertical lines denote 95% confidence limits.


Proportion Surviving
1·0
·8
·6
·4
·2
·0
0
10
20
30
40
50
60
70
Hours

saline in both experiments. One was carried out using flukes removed three days post infection, and the other with flukes removed eleven days post infection.

From the sets of survival data for flukes removed from the fish host three and eleven days post infection (table 48 A, B) the instantaneous death rates were determined. The fit of the least squares linear regressions to the natural logarithms of the two sets of data (figs. 70, 71) was significant in each case.

The analysis of variance described in chapter 6 was used to compare the regressions for the three and eleven day flukes in Cortland saline in non-sterile conditions (table 46 B, C), with those for the similarly aged flukes in sterile Hank's solution. There was found to be no significant difference between the slopes or intercepts for eleven day post infection flukes ($P > .10$) (fig. 70). There was no significant difference between the slopes of the regressions for the three day flukes ($P > .10$) but there was a significant difference between the intercepts ($P < .01$).

It is clear from these results that no improvement in survival resulted from sterile, rather than non-sterile, incubation. Although different media were used in each case, Hank's solution closely resembles Cortland saline, and both contain 1gm/litre glucose. The pH's of the media were also similar. It is hard to explain why there was a significant difference between the regressions fitted to the instantaneous death rates for three day flukes. It should be noted, however, that the three day sterile experiment was not replicated, whilst the eleven day one, where no significant difference was found, was. Perhaps, therefore, more weight should be attached to the latter result.

Using the coefficients a (intercept) and b (slope) from the regressions, predicted values for the instantaneous death rates were

calculated using the model described in chapter 3 (equation 2). The predicted values showed good agreement with the observed data (table 46B, C; table 49 A, B).

b) Survival of decaudated cercariae compared with survival of adult flukes removed from the fish host five minutes post infection.

From the survival curves for decaudated cercariae in full strength Cortland saline and tapwater (table 50 A, B; figs. 68, 69) instantaneous death rates were calculated (equation 1) (table 51 A,B). The fit of least square linear regressions to the natural logarithms of these death rates (table 51 A, B) was found to be significant.

Using the coefficients a (intercept) and b (slope) from the regressions predicted values for the instantaneous death rates were calculated using the exponential model (equation 2) and showed good agreement with the observed data (table 51 A, B).

Fig. 68 shows that the survival curves for the decaudated cercariae, and adult flukes removed from the fish host five minutes post infection, are extremely similar. The analysis of variance, described in chapter 6, was used to compare the regression previously calculated for five minute post infection flukes (table 46 A), and that for decaudated cercariae in Cortland saline (table 51 A). There was found to be no significant difference between either the slopes or intercepts of the regressions ($P > .10$ in each case). It is clear, therefore, that there is no difference between the survival of decaudated cercariae and five minute adult flukes in full strength Cortland saline in non-sterile conditions.

Figs. 68, 69 show that adults removed from the fish host five minutes post infection have become water intolerant, and, as described in section a2, 75% die in the first 1-2 hours in tapwater. The decaudated cercariae, however, do not exhibit water intolerance, and the survival curve for decaudated cercariae in tapwater (fig. 69)

FIGS.68,69.

Fig. 68

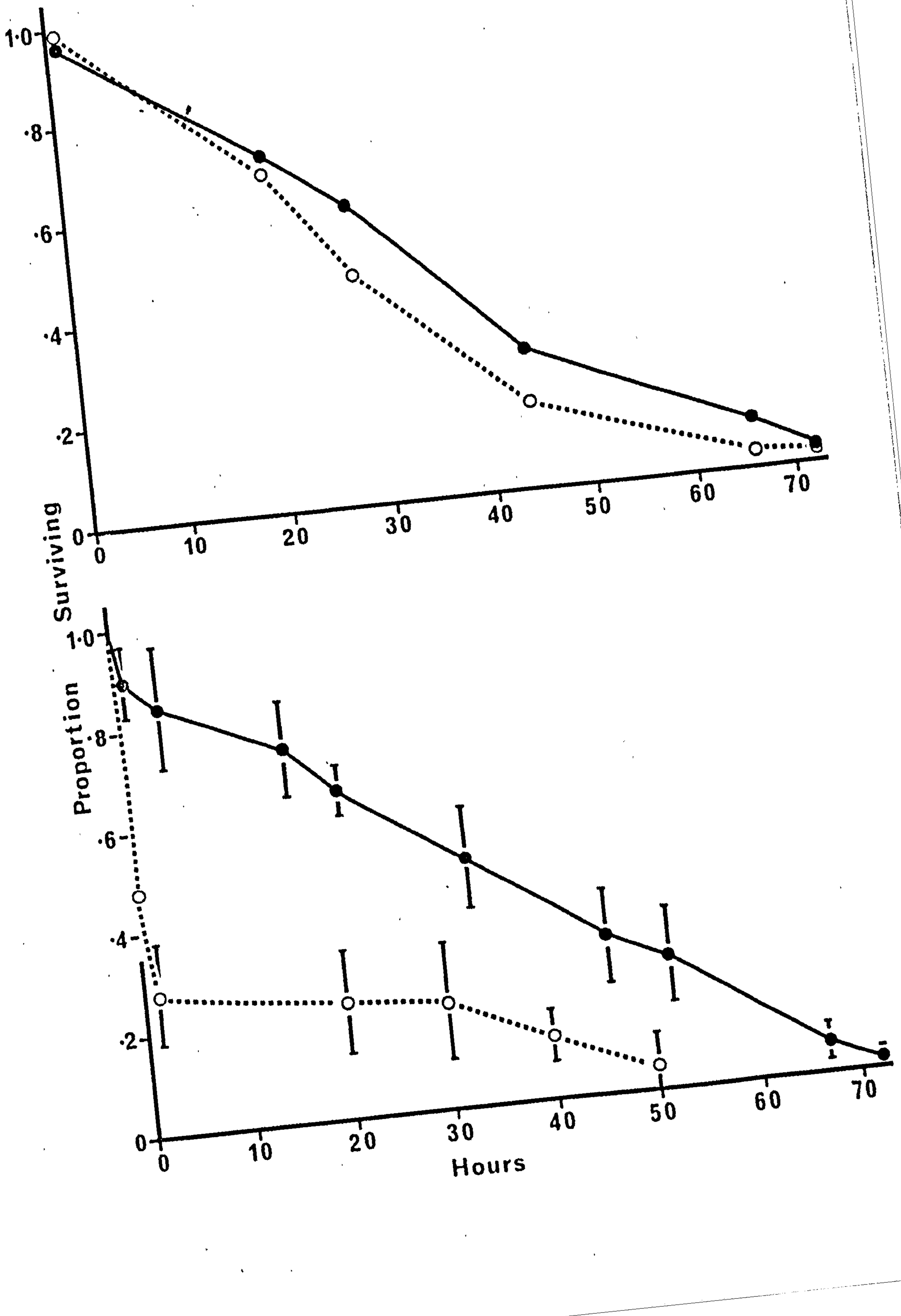
The proportion of T.patialense surviving in vitro in non-sterile conditions, in full strength Cortland saline, at a series of consecutive points in time.

1. The solid circles denote survival of decaudated cercariae.
2. The open circles denote survival of adult flukes removed from the fish host five minutes post infection.

Fig. 69

The proportion of T.patialense surviving in vitro in non-sterile conditions, in tapwater, at a series of consecutive pointsin time.

1. The solid circles denote survival of decaudated cercariae.
2. The open circles denote the survival of adult flukes removed from the fish host five minutes post infection.
3. The vertical lines denote 95% confidence limits.


1·0
·8
·6
·4
·2
0
0
10
20
30
40
50
60
70
Proportion Surviving
Hours

FIGS.70,71.

Fig. 70

Natural logarithmic transformations of the instantaneous death rates of flukes removed from the fish host eleven days post infection, in vitro at a series of consecutive points in time.

1. The solid circles show the observed data for flukes in full strength Hank's solution in sterile conditions and the solid line is a regression fitted to this data with the coefficients:

   a (intercept) = -4.0602

   b (slope) = .02551

2. The open circles show the observed data for flukes in full strength Cortland saline in non-sterile conditions and the dashed line is a regression fitted to this data with the coefficients:

   a (intercept) = -4.938

   b (slope) = .03550

Fig. 71

Natural logarithmic transformations of the instantaneous death rates of flukes removed from the fish host three days post infection, in vitro at a series of consecutive points in time.

1. The solid circles show the observed data for flukes in full strength Hank's solution in sterile conditions and the solid line is a regression fitted to this data with the coefficients:

   a (intercept) = -3.9835

   b (slope) = .02653

2. The open circles show the observed data for flukes in full strength Cortland saline in non-sterile conditions and the dashed line is a regression to this data with the coefficients:

   a (intercept) = -4.7622

   b (slope) = .03177

Fig. 72

Natural logarithmic transformations of the instantaneous death rate of decaudated cercariae in non-sterile conditions at a series of consecutive points in time.

1. The solid circles show the observed results for decaudated cercariae in full strength Cortland saline and the solid line a regression fitted to this data with the coefficients:

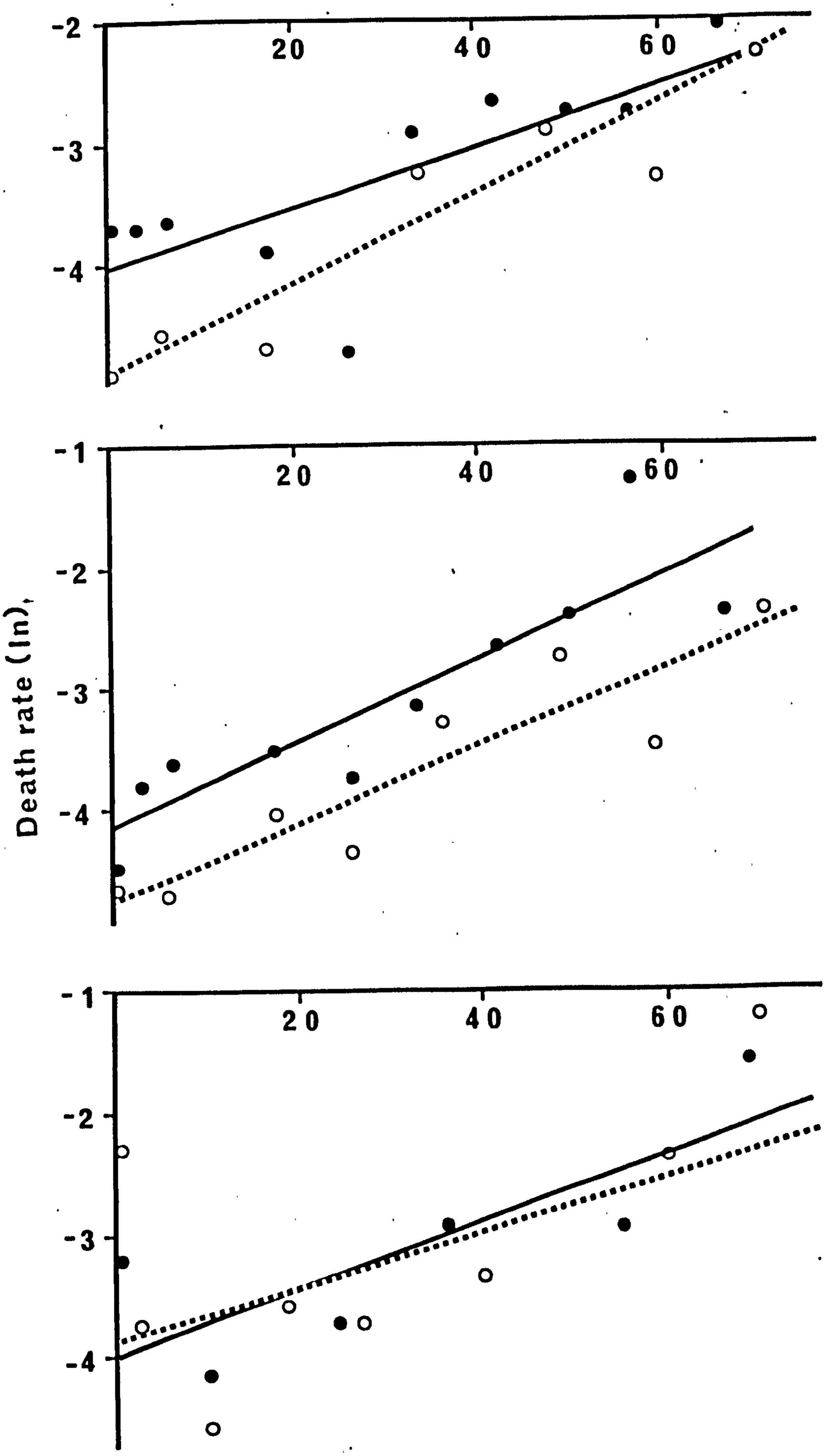
   a (intercept) = -3.9835

   b (slope) = .02653

2. The open circles show the observed results for decaudated cercariae in tapwater and the solid line is a regression fitted to this data with the coefficients:

   a (intercept) = -3.868

   b (slope) = .02318


-2
-3
-4
20
40
60
-1
-2
-3
-4
20
40
60
Death rate (ln),
-1
-2
-3
-4
20
40
60
Hours

closely resembles that for decaudated cercariae and five minute adults in Cortland saline (fig. 68).

The regressions calculated from the death rates for decaudated cercariae from tapwater and saline were compared using the analysis of variance described in chapter 6. There was no significant difference between either the slopes or intercepts ($P > .10$ in each case) (fig. 72).

The decaudated cercariae therefore do not resemble the young adult flukes in their reaction to tapwater, and have not become water intolerant. Survival in saline, however, is similar.

c) <u>In vitro egg production in full strength Cortland saline in non-sterile conditions</u>.

The production of eggs by flukes removed from the fish host eleven days post infection was measured and the effects of darkness on this egg output was determined.

Table 52 shows that the rate of egg production is extremely high in the hour after the removal of the flukes from the fish in conditions of constant light. This rate declines steadily, and by the third hour is less than the average rate per surviving fluke at 23°C for eleven day old flukes in the age dependent fecundity experiment (chapter 3). No egg production was observed after the ninth hour.

The five pots of 20 flukes placed in darkness for six hours after removal from the fish host produced a total of 87 eggs in this period. The five pots of 20 flukes placed in the light produced a total of 81 eggs.

The variance ratio obtained from the egg production in individual pots in light, and in darkness, was not found to be significant ($P > .10$). Therefore, a t-test for the comparison of the

means of two small samples with variances assumed to be equal, was carried out (Bailey, 1959). There was found to be no significant difference between the numbers of eggs per pot in light and dark ($P > .10$). There is no evidence therefore that darkness has a direct stimulatory effect on egg output on flukes in vitro.

d) Infection of Brachydanio rerio in tapwater and Cortland saline.

Twenty eight uninfected B.rerio were each placed in a small dish containing ten cercariae of T.patialense. Half the dishes contained tapwater and half full strength Cortland saline, both at 23°C. The mean percentage infection in saline was 35.7% (95% confidence limits ± 4%),, and in tapwater, 42.1% (95% confidence limits 2.03%).

These infection levels were compared using a t-test for small samples where the variances are assumed equal (Bailey, 1959). The variance ratio of the sets of data was determined and found not to be significant ($P > .05$). The square roots of the infection data were taken to transform the Poisson distributed data to a normal distribution. This was necessary because the t-test assumes normality. The means of sets of data for water and saline were found not to differ significantly ($P > .10$, 26 degrees of freedom).

The infectivity of the cercariae of T.patialense is therefore unaltered in half strength Cortland saline under these conditions. Full strength saline was not used as abnormal behaviour by B.rerio occured after short periods at this concentration.

e) Survival of cercariae in saline and tapwater.

Approximately ten freshly shed cercariae were placed in each of a series of small bowls containing tapwater or Cortland saline at 23°C. The mean proportion of cercariae surviving at a series of consecutive points in time was determined in each case.

From fig. 73 it appears that the decline in the proportion of cercariae surviving is steeper in tapwater than in saline. From

FIGS.73,74.

Fig. 73

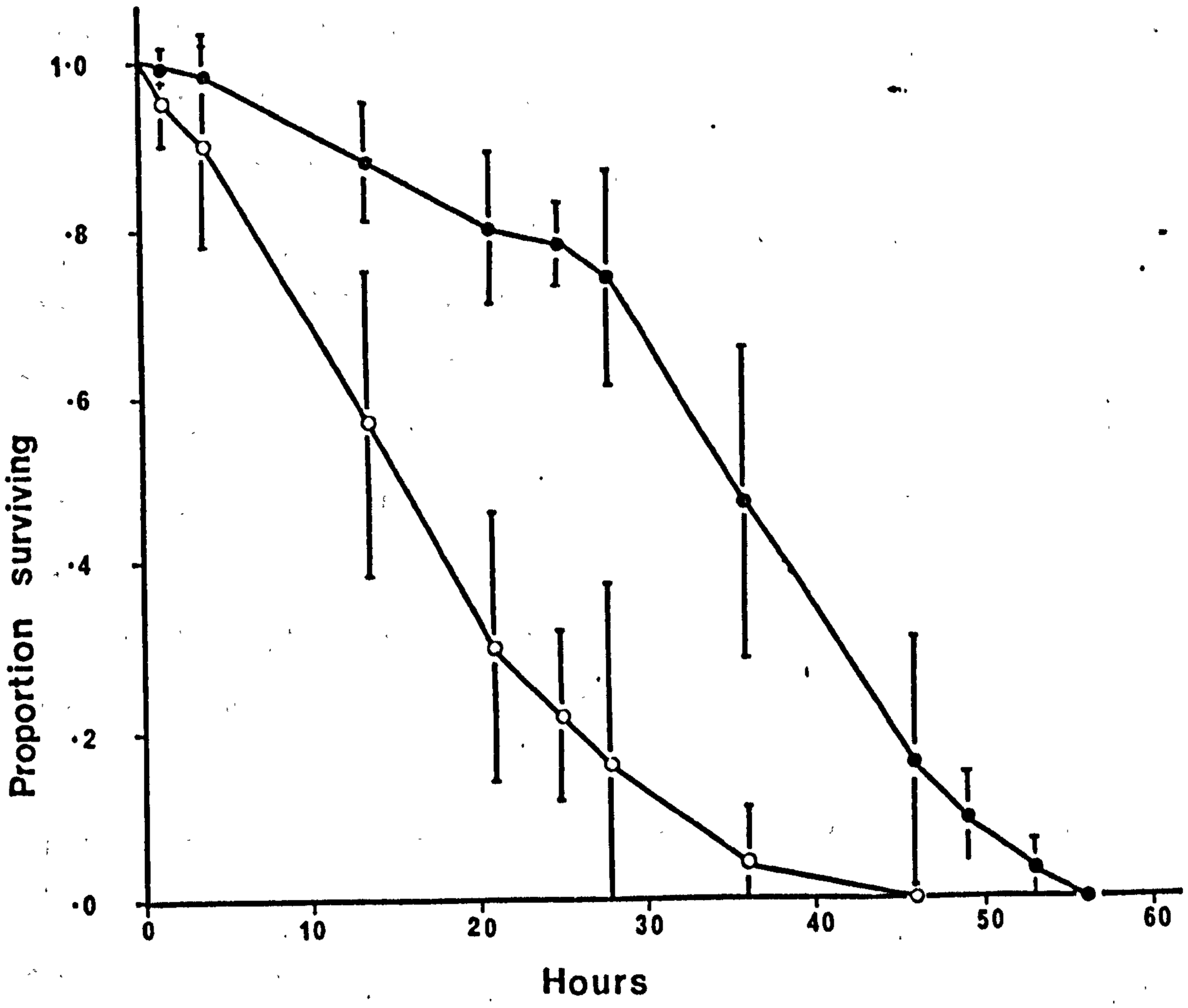
The proportion of cercariae surviving at a series of consecutive points in time.

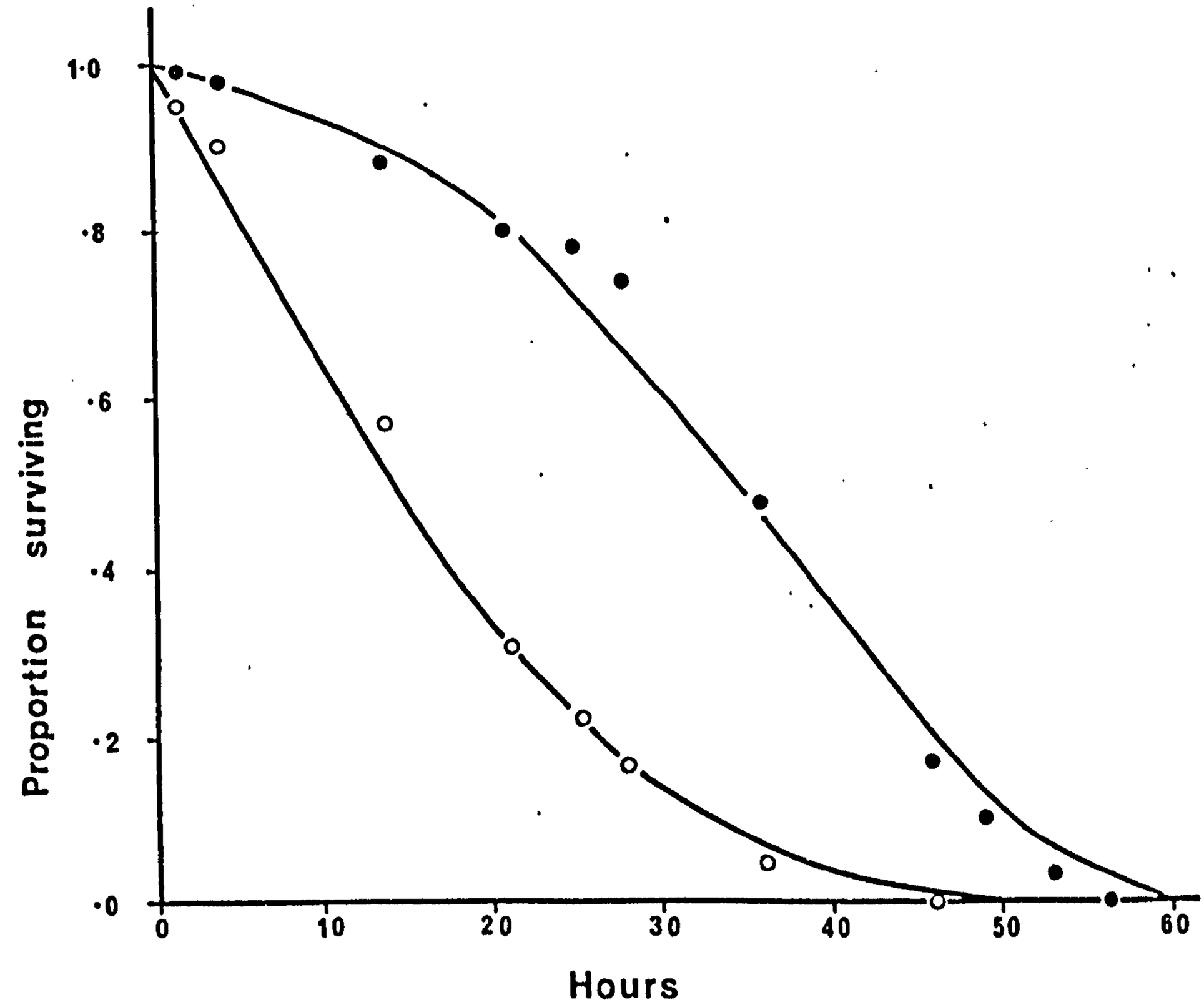
1. The open circles show the observed results for cercariae in tapwater.
2. The solid circles show the observed results for cercariae in Cortland saline.
3. The vertical lines show the 95% confidence limits.

Fig. 74

The proportion of cercariae surviving at a series of consecutive points in time, predicted by a survival model.

1. The open circles show the observed results for cercariae in tapwater.
2. The solid circles show the observed results for cercariae in Cortland saline.
3. The solid lines show the curves predicted by the exponential survival model (equation 4) using the coefficients shown in table 54.


1·0
·8
·6
·4
·2
·0
Proportion surviving
0
10
20
30
40
50
60
Hours


1·0
·8
·6
·4
·2
·0
Proportion surviving
0
10
20
30
40
50
60
Hours

the sets of data for tapwater and saline (table 53) the instantaneous death rates were determined at a series of consecutive points in time (table 54) using equation 1.

Least squares linear regressions were then fitted to the natural logarithms of these sets of data (fig. 75). For both regressions the correlation coefficients were significant ($P < .001$). The analysis of variance described in chapter 6 was used to determine whether the regressions were significantly different (table 55). The slopes were not significantly different ($P > .05$) but the intercepts were ($P < .001$). Therefore the death rate of cercariae in Cortland saline differs significantly from that in tapwater.

The coefficients a (intercept) and b (slope) from the regressions (table 54) were used to calculate predicted instantaneous mortality rates (table 54) using equation 2. The predicted proportions of parasites surviving at a series of consecutive points in time were determined using equation 4 (table 53).

FIG.75.

Fig. 75

Natural logarithmic transformations of the instantaneous death rates of cercariae, at a series of consecutive points in time.

1. The open circles show the observed death rates for cercariae in tapwater and the dashed line is a regression fitted to this data with the coefficients:

   a (intercept) = -3.6020

   b (slope) = .05137

2. The solid circles show the observed death rates for cercariae in saline and the solid line is a regression fitted to this data with the coefficients:

   a (intercept) = -5.6186

   b (slope) = .07755

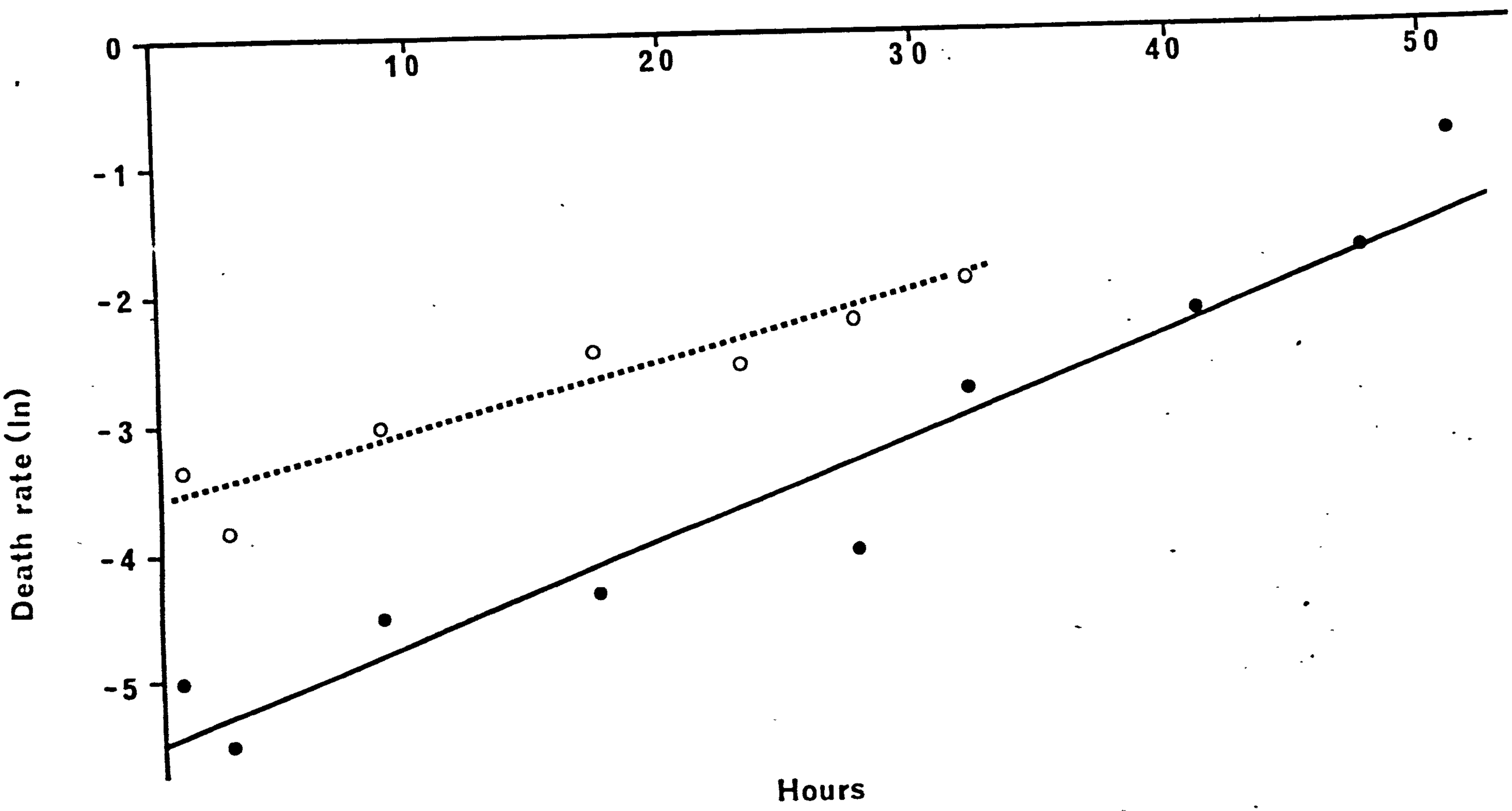

0
10
20
30
40
50
-1
-2
-3
-4
-5
Death rate (ln)
Hours

Table 44 The proportion of flukes surviving <u>in vitro</u> in non-sterile conditions at a series of consecutive points in time in a variety of culture media.

A. Flukes removed 3 days post infection

Time (hours)	100% Frog ringer	100% Cortland saline	100% Cortland saline + tap-water
.75	1.000	.994	.1000
11	.925	.905	.935
24	.750	.722	.800
28	.700	.686	.760
44	.200	.382	.430
50	.200	.259	.400
68	.100	.152	.030
74	.050	.085	.030

B, Flukes removed 11 days post infection

Time (hours)	100% Cortland saline	50% Cortland saline	10% Cortland saline
1	.993	.950	.500
11	.901	.750	.290
24	.802	.680	.210
44	.370	.230	.170
52	.246	.140	.130
68	.138	.050	.040
74	.086	.050	.040

Table 45 The proportion of flukes of different ages surviving *in vitro* in non-sterile conditions at a series of consecutive points in time in full strength Cortland saline.

A. Flukes removed 5 minutes post infection.			B. Flukes removed 3 days post infection.			C. Flukes removed 11 days post infection.		
Time (hours)	proportion surviving	95% conf. limits	Time (hours)	proportion surviving	95% conf. limits	Time (hours)	proportion surviving	95% conf. limits
1.17	.988	.014	.75	.994	.011	1	.993	.013
20	.668	.096	11	.905	.105	11	.901	.074
28	.452	.207	24	.722	.140	24	.802	.136
44	.172	.068	28	.686	.144	44	.370	.104
66	.030	.051	44	.382	.139	52	.346	.123
72	.014	.038	50	.259	.155	68	.138	.069
			68	.152	.173	74	.086	.119
			74	.085	.190			

Table 46 The instantaneous death rates of flukes of different ages *in vitro* in non-sterile conditions at a series of consecutive points in time in full strength Cortland saline.

A. Flukes removed 5 minutes post infection.				B. Flukes removed 3 days post infection.				C. Flukes removed 11 days post infection.			
Time (hours)	Instant. death rate	Rate calculated using exponential mdl.	ln. of instant. death rate	Time (hours)	Instant. death rate	Rate calculated using exponential mdl	ln. of instant. death rate	Time (hours)	Instant. death rate	Rate calculated using exponential mdl.	ln. of instant. death rate
.6	.0103	.0152	-4.5756	.37	.0094	.0086	-4.6707	.5	.0070	.0073	-4.9583
10.6	.0208	.0214	-3.8728	5.87	.0091	.0103	-4.7046	6	.0097	.0089	-4.6333
24	.0488	.0332	-3.0200	17.5	.0174	.0149	-4.0526	17.5	.0090	.0133	-4.7157
31	.0604	.0419	-2.8068	26	.0128	.0195	-4.3593	34	.0387	.0240	-3.2524
55	.0794	.0933	-2.5333	36	.0366	.0268	-3.3080	48	.0510	.0394	-2.9755
69	.1270	.1487	-2.0636	49	.0648	.0405	-2.7370	60	.0361	.0603	-3.3206
				59	.0296	.0557	-3.5197	71	.1004	.0891	-2.2986
				71	.0969	.0816	-2.3344				
a (intercept)		.01494	-4.2036			.0085	-4.7622			.0072	-4.938
b (slope)		.0333	.0333			.03177	.03177			.0355	.0355
r (correlation)			.9429				.9073				.9350
p			$<.01$				$<.01$				$<.01$

Table 47 The proportion of flukes surviving at different ages <u>in vitro</u> in non-sterile conditions in tapwater at a series of consecutive points in time.

A. Flukes removed 5 minutes post infection.			B. Flukes removed 3 days post infection.		C. Flukes removed 11 days post infection.		
Time (hours)	Proportion surviving	95% conf. limits	Time (hours)	Proportion surviving	Time (hours)	Proportion surviving	95% conf. limits
.5	.480	.123	.75	.25	1.0	.238	.119
1.5	.278	.090	11	.10	11	.078	.060
20	.226	.082	24	.05	24	.062	.041
40	.212	.091			44	.001	.022
50	.120	.042					
60	.046	.070					

Table 48 The proportion of flukes, maintained <u>in vitro</u> in sterile Hank's solution at a series of consecutive points in time.

A. Flukes removed 3 days post infection.		B. Flukes removed 11 days post infection.		
Time (hours)	Proportion surviving	Time (hours)	Proportion surviving	95% confidence limits
1.5	.983	1.5	.965	.020
5	.907	5	.885	.093
10	.793	10	.778	.075
24	.530	24	.587	.183
28	.483	28	.567	.162
38	.317	38	.322	.111
46	.183	46	.183	.108
54	.090	54	.110	.067
60	.018	60	.075	.090
74	.005	74	.013	.037

Table 49 The instantaneous death rates of flukes maintained in vitro in sterile Hank's solution at a series of consecutive points in time.

A. Flukes removed 3 days post infection.				B. Flukes removed 11 days post infection.			
Time (hours)	Instantaneous death rate.	Rate calc. using exponential model.	ln. of instant. death rate.	Time (hours)	Instantaneous death rate	Rate calc. using exponential model.	ln. of instant. death rate.
.75	.0114	.0158	-4.4741	.75	.0237	.0176	-3.7423
3.25	.0230	.0173	-3.7723	3.25	.0247	.0187	-3.7010
7.5	.269	.0200	-3.6156	7.5	.0258	.0209	-3.6574
17	.0283	.0279	-3.5474	17	.0201	.0266	-3.9070
26	.0232	.0381	-3.7636	26	.0087	.0335	-4.7444
33	.0421	.0487	-3.1677	33	.0556	.0400	-2.8717
42	.0687	.0666	-2.6780	42	.0706	.0503	-2.6507
50	.0887	.0880	-2.4225	50	.0636	.0617	-2.7551
57	.02682	.1123	-1.3160	57	.0638	.0738	-2.7520
67	.0915	.1591	-2.3914	67	.1252	.0953	-2.0778
a (intercept)		.01542	-4.1720			.01724	-4.0602
b (slope)		.03483	.03483			.02551	.02551
r (correlation)			.8870				.7533

Table 50 The proportion of decaudated cercariae surviving *in vitro* in non-sterile conditions.

A. Tapwater			B. Full strength Cortland saline		
Time (hrs)	Proportion surviving	95% confidence limits	Time (hrs)	Proportion surviving	95% confidence limits
1	.898	.067	1	.960	.047
4	.836	.117	20	.710	.093
16	.740	.086	28	.592	.200
21	.648	.046	44	.278	.108
33	.491	.098	66	.094	.109
46	.314	.091	72	.026	.045
52	.264	.091			
67	.063	.035			
73	.010	.027			

Table 51 The Instantaneous death rates of decaudated cercariae *in vitro* in non-sterile conditions.

A. Tapwater				B. Full strength Cortland saline			
Time (hours)	Instantaneous death rate.	Rate calc. using exponential model.	ln. of instant. death rate.	Time (hours)	Instantaneous death rate.	Rate calc. using exponential model.	ln. of instant. death rate.
.5	.1076	.0211	-2.229	.5	.0408	.0189	-3.199
2.5	.0238	.0221	-3.738	10.5	.0159	.0246	-4.151
10	.0102	.0264	-4.585	24	.0222	.0391	-3.735
18.5	.0266	.0321	-3.627	36	.0472	.0598	-3.053
27	.0231	.0391	-3.768	55	.0493	.1073	-3.010
39.5	.0344	.0522	-3.370	69	.2142	.1161	-1.541
49	.0289	.0651	-3.344				
59.5	.0955	.0831	-2.349				
70	.3068	.1060	-1.182				
a (intercept)		.0209	-3.868			.0186	-3.9835
b (slope)		.0232	.0232			.0265	.02653
r (correlation)			.8059				.7967

Table 52 Egg production of flukes *in vitro* in constant light, non-sterile conditions and full strength Cortland saline.

Time (hours)	Number of surviving flukes.	Egg production per hour.	Eggs per fluke per.hour.
1	122	25	.2049
2	120	16	.1333
3	120	11	.0917
4	120	10	.0833
5	118	10	.0347
6	115	4	.0348
7	114	5	.0438
8	114	1	.0088
9	114	2	.0175
10	112	0	.0000

Table 53 Proportion of parasites surviving at a series of consecutive points in time.

	Cortland saline			Tapwater		
Time (hrs) (hours)	Observed proportion surviving.	95% confidence limits	Proportion predicted by exponential model.	Observed proportion surviving.	95% confidence limits	Proportion predicted by exponential model.
1.5	.990	.020	.990	.950	.050	.960
4.0	.980	.050	.980	.900	.120	.890
13.5	.880	.070	.920	.570	.180	.590
21.0	.800	.090	.820	.300	.160	.360
25.0	.780	.050	.760	.220	.100	.250
28.0	.740	.130	.700	.160	.210	.180
36.0	.470	.180	.490	.039	.060	.058
46.0	.160	.150	.200			
49.0	.096	.050	.129			
53.0	.034	.030	.060			
56.0	.000		.029			

Table 54 Instantaneous death rates at a series of consecutive points in time.

	Tapwater			Saline		
Time (hours)	Observed instantaneous death rate.	Instantaneous death rate predicted by exponential model.	ln. of observed instantaneous death rate.	Observed instantaneous death rate.	Instantaneous death rate predicted by exponential model.	ln. of observed instantaneous death rate.
.75	.0342	.0283	-3.3760	.0067	.0038	-5.0056
2.75	.0216	.0314	-3.8351	.0041	.0045	-5.4968
8.75	.0481	.0427	-3.0345	.0113	.0072	-4.4830
17.25	.0836	.0662	-2.458	.0127	.0138	-4.3662
23.0	.0775	.0889	-2.557	.0063	.0216	-5.0672
27.0	.1062	.1120	-2.2424	.0175	.0306	-4.0456
32.0	.1412	.1411	-1.9576	.0567	.0434	-2.8700
41.0				.1077	.0873	-2.2284
47.5				.1703	.1444	-1.7702
51.0				.2595	.1895	-1.3490
a (intercept)		.02727	-3.6020		.00363	-5.6186
b (slope)		.05137	.05137		.07755	.07755
r (correlation)			.9462			.9328

Table 55 Analysis of variance to compare the slopes and intercepts of regressions fitted to ln. transformations of the instantaaneous death rates of cercariae in tapwater and saline solution.

Time (hours)	df	Σx^2	Σxy	Σy^2	Regression coefficient	Deviations from regression df	SS	MS
Within Tapwater	6	899.5	46.2109	2.6513	.05137	5	.2773	.05546
saline	9	2904.525	225.2512	20.0744	.07755	8	2.6058	.3257
						13	2.8830	.3812
Pooled (W)	15	3804.025	271.4621	22.7257	.07136	14	3.3537	.2395
				Difference between slopes		1	.4706	.4706
Between (B)	1	344.7397	-33.4637	3.4637				
W + B	16	4148.7647	237	25.9738		15	12.3207	
				Between adjusted means		1	8.9671	

CHAPTER 11.

DISCUSSION

The organisation of the discussion of the results reported in this thesis is as follows,

a) Age dependent survival and fecundity.

Age dependent survival and fecundity are discussed in the context of growth senescence immunity host size. feeding and, the unusual, ectoparasitic niche of T. patialense.

b) Temperature dependent survival and fecundity.

Temperature dependent survival and fecundity are discussed in the context of changes in the rates of chemical and physical processes, and possible ecological implications are noted.

c) Density dependent survival and fecundity.

The density dependent growth, survival and fecundity, of T.patialense, on its fish host, are discussed in the context of feeding and possible host responses to the parasite. The importance of density dependent processes in the parasites life cycle is noted.

d) The influence of light on T.patialense.

The light generated rhythm in egg production of adult T.patialense is discussed in the context of other light induced rhythms in digenean parasites.

c) The influence of ionic environment on T. patialense.

The ionic tolerances of cercarial, and adult, T.patialense, and the transformation of the cercariae to adult flukes and associated

changes in ionic tolerance, are compared with those for S.mansoni. Attempts to culture adult T.patialense of varying ages in vitro are discussed.

a) Age dependent survival and fecundity.

The phenomenon of age dependent survival in parasitic organisms on, or in, their hosts, has been ascribed to two causes; host generated immune responses (Gray, 1972) and senescence (Kennedy, 1974).

The functioning of the immune systems of mammals has been extensively investigated, and immune responses to parasites have been clearly demonstrated. For example, Smithers (1962) has shown antibody production in response to adult S.mansoni by monkeys. Such responses may be responsible for processes such as the age dependent "self cure" phenomenon, often described in nematode infections. In single infections of 5,000 Nippostrongylus braziliensis in the rat, for example, almost all worms were expelled by day 20 post infection (Mulligan et al, 1965). Immunological responses associated with trematodes can be highly complex, as demonstrated by Smithers and Terry (1967), whose transplant experiments with S.mansoni suggested that the adult worm incorporates specific host antigen to avoid the host immune mechanisms.

Understanding of the immunological systems of fish has increased considerably in the past few years, but still lags behind those of mammals. The role of host responses to parasitic infections in fish is still a matter of considerable uncertaint y.

Lester and Adams (1974) found that populations of the viviparous monogenean skin parasite Gyrodactylus alexandri increased on Gasterosteus aculatus for two weeks followed by a population decline in the subsequent two weeks. The fish were then refactory to further infection for three weeks. The decline in population numbers appeared to be related to an increase in the shedding of a layer of "cuticle", mucopolysaccharide material, secreted by the surface epidermal cells, rather than the glycoprotein mucus secreted by goblet cells (Whitear, 1970).

A cellular response has been reported to Dactylogyrus vastator on the gills of Cyprinus carpio with fish remaining insusceptible for a few weeks until the epidermis returned to its former state (Paperna, 1974).

Kennedy and Walker (1969) failed to detect circulatory antibody to the adult cestode Caryophyllaeus laticeps in Leuciscus leuciscus, despite the age dependent mortality described by Kennedy (1968, 1969). Harris (1972) suggests that this may be due to the less damaging method of attachment of the cestode in comparison with the acanthocephalan, Pomphorhynchus laevis. Leuciscus cephalus produced precipitating antibody in both the serum and the intestinal mucus, in response to natural and experiment infections, and experimental infections with the acanthocephalan. No apparent manifestation of resistance to this parasite was evident however (Harris, 1972). Similar situations have been noted in mammals, Moss (1971), for example, detected Ig A, Ig G and Ig E production in response to Hymenolepis microstoma in the mouse, yet could find no evidence of rejection of the parasite by the mouse.

Harris (1973a) found that L.leuciscus was able to produce precipitating, or agglutinating, antibody to a range of standard antigens, but failed to produce skin sensitising antibodies (Harris, 1973b). Other studies have also failed to demonstrate anaphylaxis in fish (Dreyer and King, 1948). So that Ig E, often produced in mammals with helminth infections, for example, in response to Nippostrongylus braziliensis infection (Ishizaka, Urban, Takatsu and Ishizaka, 1976), is apparently not produced by fish.

Orr, Hopkins and Charles (1969) found that in the unusual host Pungitius pungitius degenerative changes occurred in the plerocercoid of the cestode Schistocephalus solidus. The antibody located in the mucus of L.cephalus appeared to be of the Ig M type

(Harris, 1972) found in the serum, rather than the IgA type found in human mucoid secretions. Bradshaw, Clem and Sigel (1971) also found only IgM in mucus of Lepisosteus platyrhincus, a freshwater holostean, and Fletcher and Grant (1969) again found only IgM in the intestinal and surface mucus of Pleuronectes platessa. Cottrell (1977) found precipitating antibodies to the metacercariae of Cryptocotyle lingua and Rhipidocotyle johnstonei which occur in the muscle and connective tissue of P.platessa. These antibodies were of the IgM type and in the serum. There was no evidence of antibody in the cutaneous mucus although its presence here might benefit the host, as entry of the cercariae is cutaneous. Even huge infections only produced a minimal serum antibody titre and no absolute protection against recurrent infections was observed.

Nigrelli and Breder (1934) and Nigrelli (1935) found, however, that the monogenean skin parasite, Epibdella mellini, survived longer in mucus from susceptible natural host species, than from either fish with acquired immunity, or naturally immune elasmobranch species.

It should be noted however, that the latter fish might have been physiologically refactory, rather than immune in the conventional sense. Some hosts acquired a permanent, or partial resistance to the parasites after several exposures to infection.

Cyprinus carpio displayed post-invasive immunity to the ectoparasitic protozoan parasite, Ichthyophthirius sp. (Hines and Spira, 1974). These ciliates were found to swim freely in normal serum, but were immobilised in immune serum. There was also found to be a rise in antibody titre coinciding with the disappearance of the parasite. The carp were completely refactory to ichthyophthiriasis for at least eight months afterwards, but not to other parasites such as Dactylogyrus sp. The immune response appeared to act at the

level of the mucus, although there was no increase in its production and hence, secretory antibody might be involved. This accords with the results of Nigrelli, Jakowski and Padnos (1955) for infections of the epibiotic protozoans Epistylis sp. and Apiostoma sp., and Nigrelli and Ruggieri (1966) for Ichthyopthirius marinus.

Thus, to summarize, there is evidence for the presence of IgM type antibodies in the skin and intestinal mucus of some fish species in response to some parasitic organisms. The presence of such antibodies may be associated with specific immunity to, and age dependent survival of, some of these parasites. There is also some evidence for generalised surface responses to parasites, and in particular, the "cuticular shedding" of Lester and Adams (1974).

In the case of T.patialense no evidence has yet been found which indicates the presence of a host-generated immune response. The course of age dependent survival on hosts challenged with parasites at both high and low levels, showed no change from the original infections (Chapter 6). The transplantation of flukes from the existing host to a naive host showed no enhancement of survival (Chapter 6) and a trickle infection experiment over a prolonged period showed no decrease in either the rate of infection of B.rerio, or in the survival of the adult flukes (Anderson et al, 1977).

In reinfection experiments there was no evidence to suggest a decreased rate of egg production in the challenge infection. These results do not preclude the formation of antibodies to T.patialense, which may, or may not, be present in the mucus as well as the sera; but if it is present, it must be assumed that it has no detectable effect on the survival and fecundity of the parasite under the experimental conditions used here. Also there was no evidence of "cuticular shedding", a response to high levels of infestation by Gyrodactylus alexandri on the stickleback (Lester, 1972).

The fecundity of the adult flukes also shows age dependence (fig. 10). In each surviving fluke, egg production shows a rise following patency and then a decline.

The short developmental period before the commencement of egg production, 68-90 hours at 23°C, may be associated with the unusually well developed reproductive systems present in the cercariae of T.patialense. This progenesis includes the presence of motile sperm in the seminal vesicles. One part of the reproductive system, however, the vitelline glands, are only present in rudimentary form in the cercaria. In view of this the development of the vitelline glands was examined in detail to see how the time scale of development was linked with patency, and to search for a possible correlation between the growth in area of the parasite and vitelline glands and the rising phase of the rate of egg production por surviving fluke.

The vitelline glands of many trematodes contain basic proteins and phenols which are utilised during egg formation. The action of the enzyme polyphenol oxidase converts phenols to quinone,

$$\text{protein} + \text{o-diphenol} \xrightarrow{\text{polyphenol oxidase}} \text{protein} + \text{quinone}$$

The quinone produced forms cross links with the protein chains, producing the tough, quinone-tanned egg shell (Johri and Smyth, 1955).

Discrete staining methods depending on either the presence of polyphenol oxidase, basic protein or quinones have been devised. A variety of different methods for the staining of the vitelline system of T.patialense have been tested (N. A. Moloney, Zoology Dept. King's College, London, personal communication), following techniques for stainig trematode vitelline systems (Johri and Smyth, 1955).

The diazo technique based on the presence of phenolics was found to be most suitable. Several stable diazote stains are available, but Fast Scarlet Salt G.G. (Solmedia Ltd.) was found to give

the most discrete and intense stain more consistently than the Fast Red Salt B.B. used by Johri and Smyth (1955).

The results obtained using this technique show that the area of the vitellaria increases over ten times in the first 72 hours post infection. In view of this finding, and because of their essential role in providing much of the food supply and shell precursors of the eggs, it seems probable that they are indeed an important limiting factor in the commencement of egg production. Between patency and the first week post infection, the area of the vitellaria increases by a subsequent 500%. The rising phase of egg production lasts however, until 2½ weeks post infection at 23°C, whilst after the first week post infection, there is only a small increase in the area of the vitelline glands of 25%. Therefore, whilst the period of the fastest rise in the rate of egg production is correlated with rapid increase in area, the continuing rise may be explained by an increase in the thickness of the glands, or an increase in their output, or by some other factor.

Growth in area of adult flukes of T.patialense is rather limited after the increase of 50% in the first week post infection, with a further increase of only 19% up to 2.86 weeks post infection. At this point growth in width and area had reached a plateau. Although there could have been further growth in thickness, substantial growth is unlikely due to the apparently tight fit of the adult fluke under the hosts scales. Due to the generally limited growth of the adult fluke it appears that total size is not correlated with the rising phase of egg production and no other organs, apart from the vitelline glands, show obvious non-allometric growth.

The falling phase in egg production may be associated with senescence and some outward manifestations of possible senescence have been recorded in T.patialense (Chapter 8 (b)). However, after

making allowances for the flukes with these abnormalities, egg production in the remaining, apparently healthy, flukes fell almost as sharply. In some cases single old flukes with no obvious abnormalities failed to produce eggs. In the past some studies have implicated host immune responses in similar falls in parasite egg production; for example, work on S.mansoni infections (Smithers and Terry, 1965; Cheever and Powers, 1969), although no proof has been obtained for the operation of such effects on T.patialense.

No information is yet available on differences in fitness of eggs produced by flukes of different ages, although the range of egg sizes does not vary during the course of infection (D. A. P. Bundy, Zoology Dept., King's College, London, personal communication).

The underlying basis of senescence is generally little understood for all animal groups, and parasitic organisms are no exception. Generally, parasite mortality is ascribed to senescence where other causes, principally host generated immune responses, are not obviously involved. One plausible theory which would provide a mechanistic basis for senescence is that which suggests that aging is a consequence of an accumulation of somatic genetic mutations.(Emlen, 1970). Emlen argues that natural selection favours certain patterns of age dependent fecundity and mortality. He concludes that age-specific mortality should drop to a minimum prior to earliest reproductive age, and then rise with age. In some species high larval mortality may be a necessary compromise brought about by the physical nature of a selected dispersal life form. Age-specific fecundity should rise with age to a peak, which may occur at almost any age depending on the sort of organism considered, and then fall. Some organisms however tend to increase fecundity as a function of body weight throughout life, as, for example, occurs in may fish species. Traits increasing fecundity will be pushed to earlier and earlier

ages until stopped by opposing selection forces. For example, if survival is threatened, maturation may not have priority for resources. Early reproduction results in relaxed selection against mortality factors later in life. Certainly T.patialense conforms with these theoretical predictions with a high rate of larval mortality followed by extremely low mortality prior to patency, followed by a rise with age.

In the phylum Platyhelminthes there is a negative correlation between fecundity and calorific value with entoparasites at one end (high fecundity, low calorific value), and ectocommensurals at the other (low fecundity, high calorific value), with ectoparasites in an intermediate position (Calow and Jennings, 1974). The low mean egg output of adult T.patialense places it between the mean values for ectoparasites (mainly monogenean) and ectocommensurals for fecundity. It would be interesting to see if the calorific value of T.patialense also fitted this scheme, but to obtain sufficient material for calorimetry would require many thousands of adult worms and was considered to be impracticable.

The rationale for this relationship between fecundity and calorific value is that the high fecundity of entoparasites, and the low food storage in the adult parasites, is due to the continuous, superabundant and easily obtained food supply and predictable environment, so that environmental limitations on fecundity are released. Thus the adult entoparasite can produce and provision eggs without risk of over-expenditure (Jennings and Calow, 1975). This is rather different from the classical argument that high fecundity is a specific adaptation to entoparasitism.

The arguments of Jennings and Calow (1975) may, however, overestimate the stability of the entoparasitic environment by paying insufficient regard to the effects of competition and host generated immune responses. For example schistosomes need a high rate of egg

production relative to ectoparasites because only a small percentage of eggs are passed by the host, and density dependent effects on growth and fecundity are well known for entoparasites (Read, 1951; Ghazal and Avery, 1974) (Chapter 1 (e)).

It is certain however, that the fecundity of T.patialense is in line with that of organisms occupying a broadly similar environmental niche, rather than that of other adult digeneans. Calow (1973) argues that the fecundity of an organism will be controlled to the level ensuring survival of a maximal number of progeny to their reproductive age within the ecological circumstances in which it occurs. It seems likely that there are constraining environmental factors which prevent the high fecundity characteristic of entoparasitic digeneans.

An obvious possibility is differences in food supply. Halton (1967) has investigated the feeding of several species of digenetic trematodes. Gut dwelling forms, Opisthioglyphe ranae and Diplodiscus subclavatus, fed predominantly on superficial epithelial tissues and associated mucoid secretions.of their frog hosts. Gorgoderina vitelliloba and Gorgodera cygnoides feed on the bladder wall of frogs. Fasciola hepatica fed mainly on blood but also on tissues. Haematoloechus medioplexus and Haplometra cylindracea, lung flukes of frogs, fed exclusively on blood as did Schistosoma mansoni.

The mode of feeding of these parasites is suctorial and brought about by the muscular pharynx. In at least one case, H. cylindracea, this purely mechanical process is supplemented with enzymatic secretions which have a histolytic effect upon host tissue. Actual digestion is largely extracellular in all these flukes. Due to the unique position of T.patialense it is useful to look at the feeding of the Monogenea which are almost exclusively fish ectopara-

sites. The Polyopisthocotylea are mainly sanguinivorous gill parasites and digest blood intracellularly (Halton and Jennings, 1965; Lewellyn, 1954). Parasites belonging to the other sub-order, the Monopisthocotylea, occupy a range of habitats including host skin and thus are of more interest. Several suggestions have been made that they feed on host mucus and possible on epithelial cells (Bychowsky, 1975). Kearn (1963) investigated feeding in the Monopisthocotylean skin parasites Entobdella soleae and Acanthocotyle sp. and found that the pharynx was protruded to enclose a circular area of host skin. The gland cells in the feeding organ produce a proteolytic secretion which digests the host epidermis. The liquid is then pumped up by the feeding organ into the intestine.

The lack of cellular material in the intestine of T.patialense and the circular hole made in the host epidermis in the position of the pharynx provide some evidence for a method of feeding consistent with that of Monopisthocotylineans, eroding the epidermis externally, rather than that of gut dwelling digeneans.

It is difficult to make a comparison between the nutritive value of the food of T.patialense and other digeneans, but it does not seem reasonable that it would, in itself, be sufficient to account for the differences in egg production. Certainly at low parasite densities there would appear to be an abundant quantity of food for adult T.patialense. Although ectoparasitic T.patialense has a sheltered niche in some ways comparable with that of endoparasites in that it is not directly exposed to the vicissitudes of the external environment, as evidenced by its water sensitivity (Chapter 10).

The fact remains however that the total egg output through the entire lifespan of T.patialense is less than 1% of the daily egg output of a single female S.japonicum (Moore and Sandground, 1956), one of the few entoparasitic organisms for which data exists. The

fact that S.japonicum is bathed in a continually replenished supply of food, and that its wastes are instantly removed, may be important, however. Also, eggs of S.japonicum, released into the external environment, may have a lower chance of infecting the intermediate host than those of T.patialense, because unlike S.japonicum, T.patialense occupies the same aquatic environment as its intermediate host.

Although each egg of T.patialense is large, the miracidium only swims for a few hours (D. A. P. Bundy, Zoology Dept., King's College, London, personal communication), a lifespan of similar magnitude to those of many other miracidia (Oliver and Short, 1956). Therefore, there is no evidence for the production of fewer, but fitter, offspring. Although T.patialense has an enormous reproductive potential in its intermediate host, so have other digenean parasites.

Perhaps the most likely explanation for low fecundity is simply the small size of the adult parasite in comparison with many adult digeneans. As discussed previously, growth in the adult parasite is limited and may be constrainedby the small size of the parasites microenvironment under the hosts scales, although there is no evidence of size increases on hosts with larger scales.

There is a tendency for flukes to live under the scales of the mid-lateral rows where the larger scales are found, although it is difficult to establish an accurate relationship between parasite size and the area available to it under the hosts scales. This is due to the wide range of scale sizes on individual hosts and difficulty in establishing the proportion of any given scale projecting from the hosts body. Strong evidence for the constraining effect of microenvironment dimensions on adult T.patialense is provided by the effect of host size on survival (Chapter 7). Armstrong (1973) found that

scale development in B.rerio is correlated with length, and that fish under 11.5mm had no scales. Given the water sensitivity of adult T.patialense this explains the almost immediate demise of flukes on B.rerio in the 8.0 to 12.0mm length class. Scale development progresses posteriorly and anteriorly along the middle lateral band followed by a second row ventral to the first. At 14mm there are 27 scales along the lateral band and scale formation is somplete. However, in hosts in the 16.1 to 20, 20.1 to 24 and 24.1 to 28mm size classes, fluke survival increases and a plateau in 50% fluke survival time appears to exist in hosts over 28mm infected with 14 flukes per host (fig.46). This change is presumably associated with increasing scale sizes providing a fuller protection against the external environment. The precise relationship between fluke size and scale size is difficulat to ascertain for the reasons discussed previously, and is further complicated here, by the increasing size of the hosts over the duration of the experiment.

b) Temperature dependent survival and fecundity.

Most of the life processes of poikilothermic organisms respond to changes in experimental temperature. This is a consequence of the effects of temperature on the many chemical and physical processes on which they are based. In general, the rates of biological processes increase as the experimental temperature increases, up to a maximum, and then fall more or less steeply (Precht, Laudien and Harsteen, 1973). For example, Davies and Walkey (1966) found that Q_{10} values of respiration in plerocercoids of Schistocephalus solidus decrease as temperature increases up to 30°C but above this temperature they rise. The Q_{10} value is the factor by which the velocity of enzyme catalysed processes increase per 10°C rise in temperature. Q_{10} values generally decline with increasing temperature (Precht et al, 1973).

The speed of biological processes ceases to rise with temperature and begins to fall, due to protein denaturations, leading to a decline in organ and cell functions at high temperatures (Precht et al, 1973).

This type of general picture is seen in the rate of egg production which rises progressively faster to a higher peak with increasing temperature up to a high temperature (32°C) where there is a fall continuing sharply to 35°C where egg production ceases.

The relationship between temperature and survival is rather more complex however. Up to 23°C life-span and, hence, the period of egg production, increases and above 23°C it declines. Only at the highest temperatures (32, 35°C) is there any evidence of discontinuities in the coefficients a and b from the survival model, which might be indicative of protein denaturation (figs. 17, 18).

One possibility is that host immune responses affect parasite survival. There is however no evidence for this, and the

strength of the host immune response appears to rise with increasing temperature (Kennedy, 1971), which would not account for the increasing life-span with increasing temperature up to 23°C.

It is more likely that below 23°C the reduction in the rates of chemical and physical processes reduces the ability of the flukes to resist various causes of death. Possibly the rates of feeding and digestion are lowered to the extent that egg production becomes an increasing strain on food reserves, in addition to the slowing of enzymatic processes concerned with egg production such as the formation of vitelline material. At 17°C feeding may be too slow to meet both the maintenance energy requirement, and the requirements of egg production.

Above 23°C the initial faster rise and higher peak of egg production are followed by a steeper decline in the rate per surviving fluke. It is possible therefore, that below the levels at which protein denaturation may occur, the increases in the life processes of the fluke, such as egg production, may be associated with a temperature dependent aging effect. It would be interesting to look at the temperature dependent nature of the survival process more closely, by altering the temperature during the course of survival experiments, to see how far these effects are reversible. Interestingly, Clarke and Smith (1961) found a temperature dependent dying effect in males imagos of Drosophila subobscura, which was reversible, but an underlying aging effect which was temperature independent.

Another area not investigated here, is that of acclimation temperatures. Certainly in many fish species temperature tolerance is related to previous experience of environmental temperatures, due to physiological adaptations (Brett, 1956). An investigation of the survival of cercariae, as well as the survival and fecundity of adult parasites, could be carried out with differing previous temperature

experiences, by maintaining infected M.tuberculata under differing temperature regimes.

The constant temperature approach here is open to criticism. This is because it fails to answer the ecological question of how temperature, and particularly temperature fluctuations, explain some part of the distribution and abundance of T.patialense in the wild. It does, however, indicate a likely optimum temperature range for the species, and indicates possible directly temperature dependent limitations to its distribution. This, however, is conditional upon the strains of T.patialense used not having become acclimatised to 23°C. This is close to the standard temperature at which most tropical aquaria are maintained, including those from which the infected stock were obtained. It is also conditional on the wild strains of this widely distributed species all having the same temperature optimum.

It is clear, however, that the role of temperature is important in the biology of T.patialense, though there is a need for experimental studies to be validated by field studies in the natural environment of the species. Certainly in temperate regions large seasonal fluctuations in abundance of fish parasites have been reported (Anderson, 1974; Kennedy, 1971), due to either direct effects of temperature, or to temperature dependent host immune responses.

c) Density dependent survival and fecundity.

The survival and fecundity of adult T.patialense exhibit density dependence (Chapter 5). Survival is reduced where the initial parasite density exceeds 30 flukes per host (figs. 25, 26). The rate of egg production per surviving fluke shows a slight fall at the 30 fluke per host level, when compared with that of lower densities, but falls sharply at higher initial infection levels (figs. 33, 34). The growth of the parasite also shows density dependence; with mean parasite width at two weeks post infection, at an initial density of 124 flukes per host, being significantly smaller than at initial infection levels of 14 and 30 flukes per host (fig. 50).

One cause of such density dependent effects could be a non-linear increase in host generated immune responses to increasing parasite density. Jarrett, Jarrett and Urquhart (1968) found that Nippostrongylus brazilienses only provoked an immune expulsion mechanism when at least 200-250 worms were present. There is however no evidence that host immune responses, whether present or not, have any role at all in the survival of adult T.patialense, let alone a non-linear role. Even at the highest infection levels studied, a challenge infection utilising an identical number of cercariae showed no decrease in parasite survival, and no significant decrease in the initial establishment of flukes over the primary infections.

The density dependent growth of the parasites could be a cause of reduced survival and fecundity at high initial parasite density levels. In the absence of apparent immune effects, the most obvious mechanism producing this "stunting", is some form of intra-specific competition.

Each fish host in the 28-32mm size class has in excess of 400 scales, some of which may provide a better microenvironment for the adult parasite than others. Certainly there is a considerable

range in the size of scales on each host, and possibly at high densities, more flukes are forced to occupy the poorer environments. Also it is obvious that the microenvironment of some scale recesses is likely to be disturbed by the flexion of the fishes body during swimming, particularly those of the tail. Such disturbances could lead to ionic disturbances or the dislodgement of the parasite. Another possible cause of dislodgement is the increase in fluke-fluke contacts with increasing density. Due to the photonegative response of the flukes in response to the microscope light during examination of flukes on anaesthetised hosts, it is difficult to assess the extent of natural scale to scale movements by adult flukes, although they certainly occur. An initial reason for these movements may, in fact, be to seek fluke to fluke contacts for cross-fertilisation, which from the reproductive anatomy of T.patialense appears likely to occur. Single fluke infections showed however, that lack of cross-fertilisation has no obvious effect on egg production. The other likely cause of scale-scale migrations is to move to new feeding sites, which, judging from the density dependent growth, might be in short supply at high parasite densities. Fish epidermis, however, does heal and regenerate rapidly following superficial damage. The epidermal cells round the margin of the wound secrete mucus and there is an invasion by lymphocytes followed by a migration of epidermal cells which close the wound within 24 hours (Oosten, 1957).

A feature of density dependent survival in T.patialense is that even after the population has fallen to a level where no density dependent mortality would normally occur, i.e. below that of the 30 fluke per host infections at an equivalent time post infection, an increased death rate is still observed. This indicates that the experiences of T.patialense during early growth have an important effect subsequently.

Fig. 32 shows, however, that after two weeks post infection in the 145 flukes per host class, and four weeks in the 72.4 flukes per host class, the proportion of weekly mortalities attributable to density dependent factors, steadily decreases and the proportion attributable to age dependent factors, increases. By the final two or three weeks of the infection no influence of density dependent factors can be detected.

Following density dependent reductions in egg production in the 145 parasites per host class, there is a rise in the rate of egg production per surviving fluke, until egg production equals that of the 14 fluke per host class by five weeks post infection. So whilst the effects of density dependence at very high initial parasite densities reduce the population per host below that of populations unaffected by density dependent effects, there is an apparent recovery in fecundity. In the late stages of the infection the effects of density dependence are either swamped by the increasing age dependent mortality, or have been lifted. Due to this reduction in density dependence, the maximum duration of heavy infections is only reduced by one or two weeks, compared with the lower levels.

If the density dependence is due to intra-specific effects amongst the parasites, these obviously affect the flukes in a way that reduces their survival over a prolonged period after the removal of these effects. This points to some deleterious consequence of density dependent growth, rather than physical dislodgement of the flukes as the underlying mechanism for density dependent growth and fecundity.

An alternative hypothesis to explain density dependence, is that there may be a non-immune host response which operates on T.patialense in a manner which increases in a non-linear fashion with increasing parasite density. Such a response would have to continue to operate for a period after parasite numbers had been reduced to

levels where this response would not normally be provoked. Such a response would presumably involve host surface changes, such as increased mucus production, or the "cuticular shedding" observed by Lester and Adams (1974). The former is hard to investigate in a quantitative manner, but does appear to occur to a limited extent, possibly in connection with epidermal damage by the parasite. On a very limited number of occasions, small surface haemorrhages were observed on heavily infected hosts, although these soon healed. No "cuticular shedding" was ever observed.

The presence of parasites under scales causes them to be raised slightly above the fishes surface. In some heavily infected fish the scales did appear to be raised further away from the fishes surface than on normal infected fish. This effect, which proved impossible to quantify, may have been simply due to the presence, and movements, of so many flukes, or it may have been some type of host response caused by the feeding damage of the parasite to the hosts epidermis. This raising of the hosts scales may have increased the chance of dislodgement of the parasites, and increased the degree of harmful contact of the parasites with the external environment.

There is evidence that T.patialense can damage its host more seriously, as on a small number of occasions, host mortality occurred within an hour of heavy infections (Dr. P. H. Whitfield, Zoology Dept., King's College, London). It is difficult to postulate a mechanism for this rapid lethality, apart from some sort of osmotic shock, possibly caused by the commencement of feeding of the parasites. It is not known definitely, however, how soon after attachment feeding commences. No host mortality was observed amongst the heavily infected hosts from any cause at any time during the experiments described in Chapter 5.

It is interesting to note that despite the effects of both

density dependent survival and fecundity, reducing average total egg output per fluke during the course of infection, the increasing number of flukes compensates for this up to high initial density levels. Thus, the highest cumulative egg output per host during the course of an infection occurs in the 72.4 fluke per host class. At the highest initial parasite densities however, total cumulative egg production per host is reduced below this level. This accords with the results for hymnolepid infections (Hesselberg and Andresson, 1975; Ghazal and Avery, 1974), rather than those for the nematode Ostertagi ostertagi where faecal egg counts ran a stereotyped course which bore little relation to parasite numbers except at very low parasite densities, implying an upper limit to egg production imposed by the parasite populations environment.

Anderson et al (1977) argue that density dependent processes must be present to regulate population growth in complex parasite life cycles, as in other life cycles, because the complexity of the life cycles per se does not necessarily confer stability on the system. These authors also note that the numerous distinct parasite and host populations present in digenean life cycles, lead to the presence of large numbers of rate parameters which create many opportunities for density dependent processes to operate.

It is clear that survival and fecundity of adult T.patialense on B.rerio are examples of such processes. There is also evidence for parasite induced host mortalities. The existence of more density dependent processes in the life-cycle is likely. Firstly, multiple miracidial infections are unlikely to be associated with concomitant increases in cercarial output by M.tuberculata. This is because the multiplication of the parasite in its intermediate host should enable even a single miracidial infection to fully exploit the food resources available in the snail. Secondly, the survival and reproduction

of M.tuberculata may be affected adversely by infection with T.patialense, due to the demands made on the host by cercarial production. Thirdly, the predatory activity of the fish host on the cercariae of T.patialense is important, due to its influence on the cercarial density and, hence, the rate of infection. Preliminary observations on B.rerio feeding on cercariae suggest that it exhibits a type three functional response (Holling, 1965). This is typical of relatively sophisticated predators, with the fish spending an increasing proportion of its time feeding on the prey as its density increases, up to an upper asymptote. This results in a sigmoid response curve (Anderson et al, 1977). Such responses are capable of stabilising interactions between predator and prey populations (Hassell et al, 1976b).

It is to be hoped that further experimental studies on aspects of the life cycle of T.patialense will provide insights into the dynamical properties of complex host-helminth parasite interactions.

d) The influence of light on T.patialense.

Light can play an important role in digenean life cycles by both directly affecting occellate free living stages, and directly affecting the parasitic stages on, or within, their hosts.

There are, for example, a number of accounts of diurnal emergence rhythms of cercariae. Asch (1972) found that emergence of the cercariae of Schistosoma mansoni from the snail Biomphalaria glabrata was light induced. This was due to the effect of light itself, rather than changes in temperature within the snail. It was proposed that the cercariae were responding to light induced host rhythms. Olivier (1951) showed that cercariae of Schistosomatium douthitti emerged mainly in the dark, and that by keeping the snail hosts in the dark during normal daylight hours this pattern can be reversed. Anderson, Nowosielski and Croll (1976) found that the emergence of Trichobilharzia ocellata from Lymnaea stagnalis from snails acclimatised to 12 hours light, 12 hours dark periodicity, showed a marked diel pattern. This pattern was reversed when the light-dark regime was reversed. It proposed that this emergence pattern is governed by light influenced activity patterns in the snail.

These rhythms are of biological significance in ensuring the presence of free living cercariae at times of maximal definitive host contact with, or activity in, their aquatic environment.

A similar light generated circadian rhythm is present in the egg production of T.patialense. Diurnal rhythms in egg production have also been recorded in Schistosoma sp.. McMahon (1976), for example, found a circadian rhythm in the excretion of the eggs of Schistosoma haematobium. Peak egg production occurred in the late morning and early afternoon, with a partial reversal in the rhythm in night shift workers. It is suggested that the rhythm is related to the light generated activity patterns of the human host. The

mode of action may be through chemical or physical stimuli triggering enzyme release by the fully developed miracidia in the eggs. This would result in the extrusion of the ova nearest the bladder wall.

There is little chance of an actual rhythm in oviposition however, as it takes about six days for eggs to migrate to the bladder of the host. The biological significance of the rhythm is that the release of the eggs at a time when host contact with the water is greatest, will increase the likelihood of the miracidia infecting the aquatic intermediate host.

It was found that all egg production in adult T.patialense removed from the fish host ceased after ten hours post removal (table 52). There was no significant difference between the number of eggs produced in light and darkness. However, the short period for which egg production could be maintained in vitro allows little weight to be attached to these results. Only if egg production could be maintained for, say, 48 hours, could the presence, or absence, of an inherent light rhythm in egg production be established. If such an inherent rhythm were present, the occellate adult fluke would be reacting directly to light. The adult fluke can certainly respond directly to light, moving away from the light of the microscope whilst the anaesthetised host is under examination.

In the light of the previously quoted examples it is perhaps more likely that the rhythm of egg release from the infected host carrying T.patialense is linked to diurnal host activity rhythms. Nothing is known about such rhythms in B.rerio however.

The marked rhythm in egg production (fig. 58) in T.patialense appears to be generated by the actual rate of egg formation by the flukes.

No temporarily varying accumulation of eggs was observed in the flukes or under the hosts scales. It is not easy to suggest what

biological significance the release of the eggs during darkness might have. A substantial developmental period is needed before the miracidia are fully developed and hatch (Sim, 1972). It is possible that upon release from beneath the hosts scales the eggs are vulnerable to predation whilst they sink to the bottom. Certainly eggs are found, on occasion, embedded in the faeces of their fish hosts and have almost certainly been eaten by them.

e) Influence of ionic environment on T.patialense.

The cercariae of T.patialense survive significantly better in full strength Cortland saline than in tapwater (fig. 75), whilst in 50% Cortland saline, infection was not significantly different from that in tapwater (full strength Cortland saline has an ionic concentration 25% sea water). These results are not surprising when seen in the biological context of the environmental range of T.patialense. This host species is found in fresh water tanks, lakes, canals and streams as well as brackish water and mud flats. The snail has a salinity tolerance of up to 40% sea water (Anantaraman, 1972). Also other members of the genus Transversotrema have marine and brackish water hosts (Velasquez, 1958; 1961; Manter, 1965).

A similar situation exists for the cercariae of S.mansoni, with survival being enhanced in .01-.03 M NaCl solution and .01 M Tris- H Cl buffer (Asch, 1975). Chernin and Bower (1971) found that cercariae and miracidia of S.mansoni could survive, and remain infective, in 25% sea water even though a common intermediate host, Biomphalaria glabrata is rather intolerant to salinity. It seems possible that many cercariae can survive in a range of ionic conditions as Stunkard and Shaw (1931) found that a wide range of cercariae maintained normal activity for considerable periods of time in solutions containg 12½% and 25% sea water. A possible explanation for the enhanced survival noted for S.mansoni and T.patialense is that less energy is required to maintain the internal ionic constitution of the cercariae when there is a smaller osmotic gradient between the cercariae and the aqueous environment. Such considerations may be of great importance in governing the longevity of cercariae due to their finite energy reserves.

If the cercariae of T.patialense successfully infects B.rerio, a change takes place in the first five minutes post infection

with the young adult flukes becoming extremely water sensitive (fig. 63). The change is almost completed in this short period immediately post infection with only a slight further increase in water sensitivity at three, and eleven, days post infection.

This process bears similarities to the transformation of S.mansoni cercariae to schistosomula. In S.mansoni there is an increase in water sensitivity associated with an increase in permeability within minutes of tail loss (Howells, Ramalho-Pinto, Gazzinelli, de Olivera, Figueiredo and Pellegrino, 1974; Ramalho-Pinto, Gazzinelli, Howells and Pellegrino, 1975).

The transformation of the cercariae of T.patialense to the adult fluke appears to a simple process of tail loss. In S.mansoni the process if rather more complex. Proteolytic secretions are released from the acetabular glands (Howells et al, 1974) and the fibrillar glycocalyx, or surface coat, is shed by the cercariae (Ramalho-Pinto et al, 1975). The apparent lack of these processes in T.patialense is probably associated with the ectoparasitic microenvironment of the adult fluke. This obviates the necessity for penetration glands, and the need to expose receptor sites for binding host antigen (Howells et al, 1974) to prevent destruction by host immune responses during migration.

The actual mechanism of tail loss in S.mansoni appears to be a simple mechanical trauma, effected by the movement of the cercarial tail acting against the resistance of the immobile body (Howells et al, 1974). On the evidence of the rapid beating of the tail of T.patialense cercariae immediately prior to tail loss (Whitfield, Anderson and Moloney, 1975) the same is probably true for this species. One possibility, therefore, is that the rapid increase in water sensitivity, in both cases, may be due to the lesion resulting from tail loss. However, this lesion quickly heals in S.mansoni, whilst the

water sensitivity is not reversed, and it is suggested that subsequent increases in water sensitivity are correlated with body surface changes (Howells, Gerken, Ramalho-Pinto, Kawazoe, Gazzinelli and Pellegrino, 1975) and the loss of the surface "coat" (Howells et al, 1974).

Despite the apparent lack of coat loss in adult T.patialense it is interesting that, like the adult entoparasite S.mansoni, they are water sensitive. This suggests that despite the ectoparasitic niche of adult T.patialense, the ionic status of the microenvironment may be closer to that of host body fluids, than to that of the external medium of its freshwater definitive hosts, which is rapidly fatal. Figs. 60 and 61 do show, however, that adult flukes survive as well in Frog ringer solution as in the Cortland saline which has been used in the culture of fish cell lines (Wolf, 1963). Survival is almost as good in 50% Cortland saline as in the full strength solution, although survival in 10% Cortland solution is poor. Thus, although a relatively high ionic concentration appears necessary for prolonged survival, it need not exactly correspond with that of the hosts body fluids. Nevertheless, this ionic requirement helps to explain the apparent ectoparasitic niche of T.patialense in the context of a taxon of parasites almost all of which are ectoparasitic. Other digeneans do indeed form metacercarial cysts on the surface of fish, and it is possible that in evolutionary time some ancestor of the transversotrematids found the ionic conditions under the scales of a freshwater, or marine, host, suitable for further development resulting in the loss of the previous definitive host from the life cycle of the ancestral transversotrematid.

The survival of adult flukes may be limited in vitro by the exhaustion of food reserves. This has been suggested as a cause of the markedly age dependent survival of the cercariae of T.patialense, with a progressive decrease in the glycogen reserves in the tail

(Whitfield et al, 1975). The survival curves for the cercariae and adults removed from the host five minutes post infection and maintained in Cortland saline, are, in fact, extremely similar. It would be interesting to compare the survival curves of such adults obtained from cercariae of differing ages, rather than just the recently shed ones utilised in the experiment. Such experiments would determine whether food reserves in the cercarial body, as well as the tail, have run down during swimming. Flukes removed from the host three and eleven days post infection survived *in vitro* significantly longer than the young adult flukes (fig. 65); which could be explained on the basis of larger food reserves in the former worms. Survival of adult flukes *in vitro* in non-sterile conditions was not significantly greater than in non-sterile conditions (figs. 66, 67, 70, 71), eliminating bacterial, or fungal, attack as a cause of mortality. Thus it would appear that more sophisticated techniques, possibly involving the use of biphasic media enabling feeding to take place, would be necessary for more prolonged culturing and the maintenance of egg production *in vitro*.

Ramalho-Pinto, Gazzinelli, Howells, Mota-Santos, Figueiredo and Pellegrino (1974) and Colley and Wikel (1974) describe methods for the production of schistosomules *in vitro* by mechanical removal of the cercarial tail, and a modified version of the Ramalho-Pinto method was used to decaudate cercariae of *T.patialense*. The schistosomules produced using this technique appear to more closely mimic naturally produced schistosomules than schistosomules produced by incubation of cercariae in rat serum (Brink, McLaren and Smithers, 1977), and produce viable adult worms. Mechanically produced schistosomules also showed development of water sensitivity (Ramalho-Pinto et al, 1974). However, with *T.patialense*, the mechanically decaudated cercariae did not become water sensitive (figs. 68, 69, 72). If, as already

suggested, tail loss *in vitro*, is also a simple mechanical process, this is evidence for some other change, possibly in the parasite surface, brought about presumably by contact with the host surface, which requires further investigation. Certainly in the case of *S.mansoni*, permeability changes resulting from the tail-loss lesion are only partially responsible forthe water sensitivity, and here there are well documented surface changes (Howells et al, 1975, 1974).

It was not possible to determine whether these mechanically produced "adults" were viable on the definitive host. This was because they proved harder to manipulate than adult flukes removed from fish hosts, because it was hard to attach them to the microhook.

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APPENDIX 1.

Distribution of the Transversotrematidae.

Members of the genus Transversotrema are found in fishes of fresh, brackish and sea water. The intermediate hosts of T.haasi and T.licinum are not known, though the definitive hosts were both marine fish, as is the definitive host of the only known member of the genus Prototransversotrema. All other records of both intermediate and definitive hosts for the genus were from fresh water. A full list of both the definitive and intermediate hosts the Transversotrematidae is given in table 56.

The range of M.tuberculata extends from fresh water tanks, lakes, canals, streams and creeks of rivers to brackish water and mud flats. Its tolerance to salinity extends up to 40% of full strength sea water (Anantaraman, 1972) so it is possible that some of the natural definitive hosts of T.patialense could be euryhaline, or brackish water, fish.

The family Transversotrematidae has a very wide distribution, encompassing Africa, India, South East Asia, Australia and the Pacific Ocean. All the intermediate hosts known for T.patialense are in the genus Melanoides, there being four records from India in M. tuberculata, and one from M.scabra. The sole African report is from M.anomala. Judging from the wide range of M.tuberculata, T.patialense may have a far wider distribution than currently recorded.

The possibility also exists that some of the other species reported, particularly from M.tuberculata, may be synonymous with T.patialense. Certainly the size of the adult or cercaria is not a reliable feature on which to base classification, as the methods of fixation are rarely reported in the literature and no statistical proof of the significance of size differences is supplied.

FIG.76.

Fig. 76

The geographical distribution of the Transversotrematidae.

1. The solid circles denote Transversotrematids with Melanoides tuberculata as an intermediate host.
2. The stars surrounded by open circles denote Transversotrematids without M.tuberculata as an intermediate host.
3. The numerals refer to table 56.

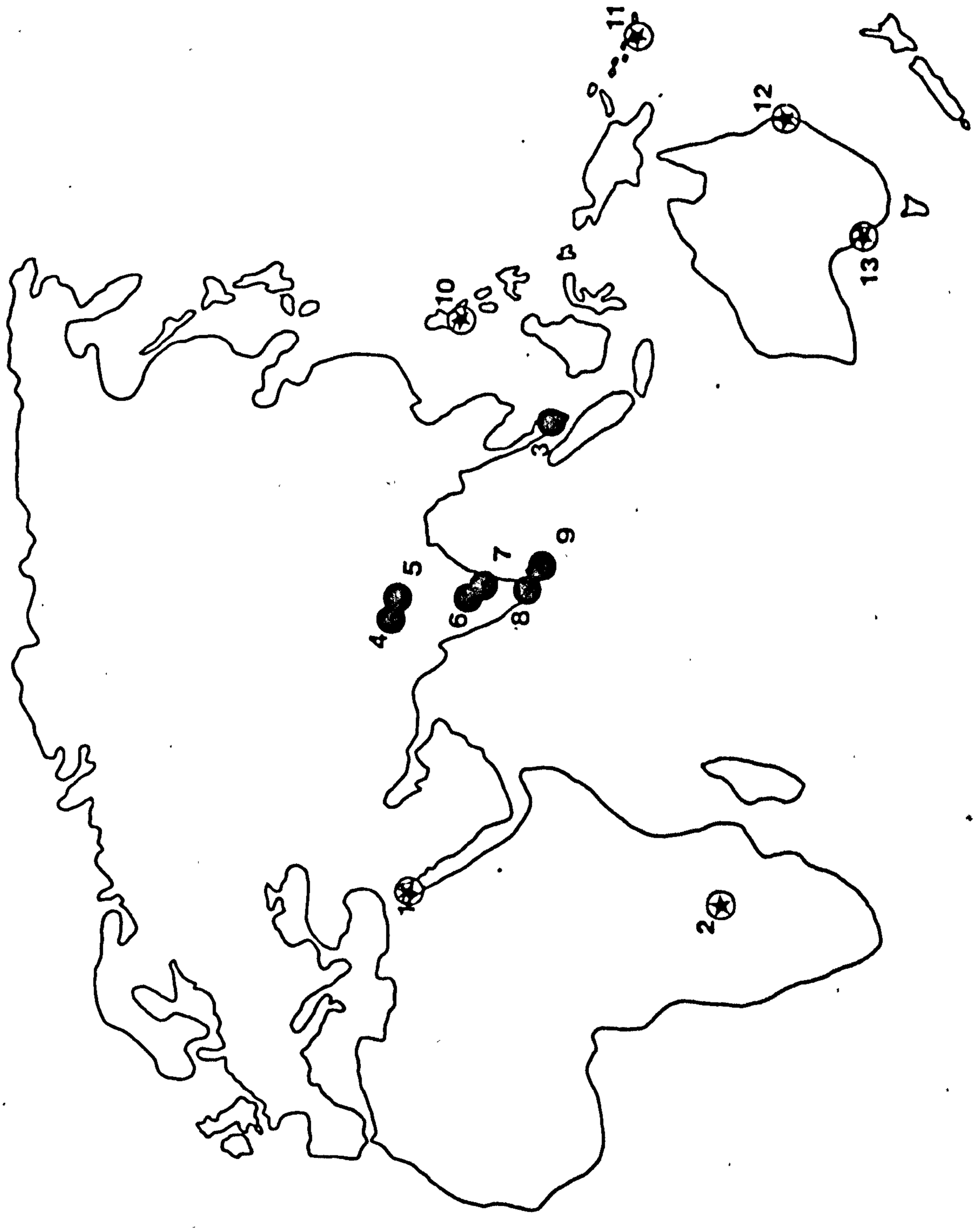
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Table 56 Geographical distribution of the family Transversotrematidae and its intermediate and definitive hosts.

* experimental host only.

Parasite	Definitive hosts	Intermediate hosts	Location	Fig.76	Source
T.patialense		M.tuberculata	Punjab	5	Soparkar (1924)
T.patialense	Macropodus cupanus	M.tuberculata	Ceylon	9	Crusz (1960)
T.patialense		M.tuberculata	Madras	6	Anantaraman (1948)
T.patialense	Esomus danricus		Waltair	7	Rao & Ganapati (1967)
	Panchax panchax				" " "
T.patialense		M.anomala	Belgian Congo	2	Brien (1954)
T.patialense	Brachydanio albineatus*				Whitfield & Wells (1973)
	Mollinesia sp.*				" " "
	Hyphessobrycon flammeus*				" " "
T.patialense	Brachydanio rerio*	M.tuberculata			Whitfield et al (1975)
T."malayan sp."	Trichogaster pectoralis	M.tuberculata	Malaya	3	Sim (1972)
	Achrosochellus sp.				"
	Tilapia mossambica*				"

cont.

Table 56 continuation

Parasite	Definitive hosts	Intermediate hosts	Location	Fig.76	Source
	Trichopsis vittatus*				Sim (1972)
	Rasbora elegans*				"
	Puntius hexazoon*				"
	Anabas testudineus*				
T."malayan sp."	Trichogaster trichopterus		Malaya	3	Betterton (pers.comm.)
	Rasbora sumatra				"
	Aplocheilus panchax				"
	Brachydanio albolineatus				"
	Betta pugnax				"
T.chackai		M.tuberculata	Kerala	8	Nadakal et al (1969)
		M.scabra			" "
T.soparkari	Puntius sophore	M.tuberculata	Lucknow	4	Pandy (1970)
	Puntius chola				"
	Channa punctatus				"
	Nandus nandus				"

cont.

Table 56 continuation

Parasite	Definitive hosts	Intermediate hosts	Location	Fig.76	Source
	Cirrhinus reba				Pandy (1970)
	Amphypharyngodon mola				"
T.haasi	"fish"		Red Sea	1	Wittemberg (1944)
T.laruei	Lates calcarifer	Thiara riquetti	Phillipines	11	Velasquez (1958)
	Mollinesia latipinna				"
	Scatophagus argus*				"
	Mugil sp.*				"
	Tilapia rossembica*				"
	Anodontostoma chacunda*				"
	Megalops cyprinoids*				"
	Therpon argentus*				"
T.licinum	Scorpius sp		Queensland	12	Manter (1965)
	Microcanthus stigatus				" (1970)
T.koliensis		M.tenebra ?	Solomon Islands	10	Oliver (1947)
P.steeri	Aldrichetta fosteri	Cerithid snail?	South Australia	13	Angel (1969)

J. Anim. Ecol. (1977), 46, 555–580

AN EXPERIMENTAL STUDY OF THE POPULATION DYNAMICS OF AN ECTOPARASITIC DIGENEAN, *TRANSVERSOTREMA PATIALENSE*: THE CERCARIAL AND ADULT STAGES

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INTRODUCTION

At present very little is known about the biological and physical processes which influence and control the dynamics of helminth parasite populations. This gap in the ecological literature is partly due to the complexity of many parasite life cycles which may involve more than one species of host and many distinct parasite populations. The numerous population variables and rate parameters involved in host-parasite interactions not only create difficulties in field and experimental work, but also hinder theoretical studies of a mathematical nature aimed at gaining insights into the dynamical properties of such systems. Further problems are raised by the intimacy of the relationship between host and parasite, which makes observation and measurement difficult, particularly in the case of endoparasitic organisms.

The work reported in this present paper concerns some preliminary results from an experimental investigation of the population dynamics of an ectoparasitic digenean, *Transversotrema patialense* (Soparkar 1924), under laboratory conditions of constant temperature and dark-light regimes. The general aim of the overall study of this parasitic species, is to elucidate the functional forms of the many population rate parameters, such as birth, death and infection rates which control the dynamics of a complex helminth parasite life cycle. Attention is initially focused on populations of two particular developmental stages in the parasite life cycle, a free-living infective larva, the cercaria, and the adult parasite which is ectoparasitic on the surface of tropical freshwater fish species.

LIFE CYCLE

The family Transversotrematidae is a taxon of digeneans with ectoparasitic adults. Qualitative descriptions of certain aspects of the life cycles of these parasites have been made by Velasquez (1961), Crusz, Ratnayake & Sathananthan (1964) and Rao & Ganapati (1967). Two hosts are involved, a fish and a melanid gastropod. Within the latter, rediae of the digenean give rise to biocellate, furcocercous cercariae. When released from the snail these cercariae swim and directly infect a fish by attaching themselves to its outer surface (Whitfield, Anderson & Moloney 1975). Attached cercariae develop into adults that inhabit the recesses under the scales of the fish host. Here they produce large, tanned eggs that are shed into the aquatic habitat of the fish. A small ciliated miracidium develops within the egg and eventually hatches and adopts a free-swimming mode of life. This larval stage penetrates the snail intermediate host and initiates multiplicative larval development via at least two redial generations, to the free-swimming cercarial larvae.

Transversotrema patialense is highly host specific in its larval stages, development probably only occurring in the melanid gastropod, *Melanoides tuberculata* (Müller). In contrast, the cercarial stage is capable of infecting and successfully developing to the adult stage on a variety of fish species (Whitfield & Wells 1973). This lack of host specificity can be demonstrated under laboratory conditions but has also been noted in natural populations of the parasite in Malaysia (personal communication, Dr C. Betterton,

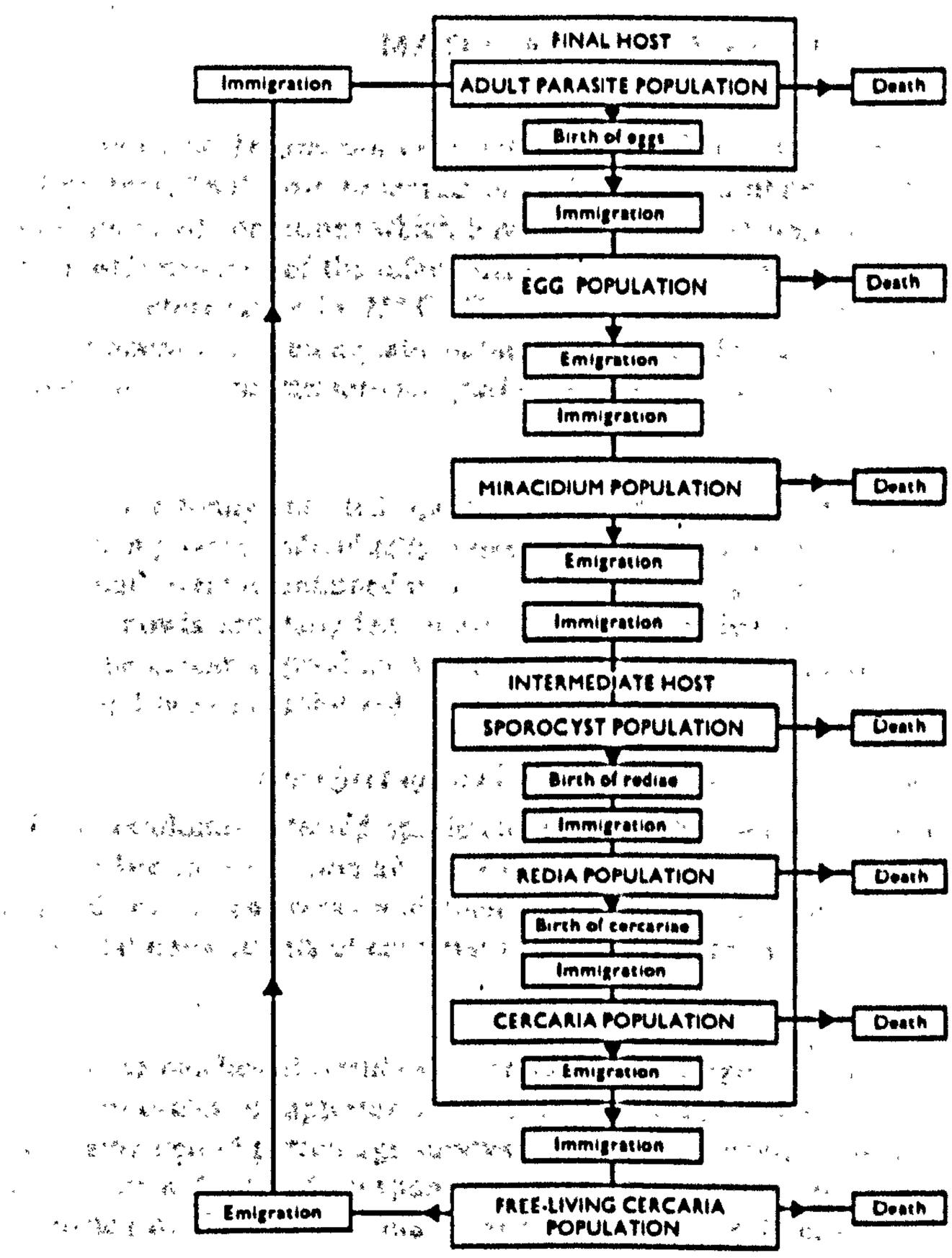

FIG. 1. Diagrammatic flow chart of the population processes involved in the life cycle of a typical transversotrematid.

University of Malaysia, Penang, Malaysia). In the experimental work reported in this paper, adult flukes were maintained solely on the Zebra Danio (*Brachydanio rerio* Hamilton-Buchanan).

The gross features of the population dynamics of the life cycle of *Transversotrema patialense*, are illustrated in a flow chart (Fig. 1). This type of representation demonstrates the various population processes responsible for controlling the flow of parasites through

the life cycle, and emphasizes the complexity of such a system. The processes controlling the population dynamics of the intermediate and final hosts are not included in Fig. 1.

The biology and life cycle of *T. patialense* manifest a series of favourable features which make this helminth peculiarly suitable for laboratory based studies of population biology (Anderson & Whitfield 1975; Whitfield, Anderson & Bundy 1977). Its ecto-parasitic microhabitat (in the recesses beneath the fish scales) makes parasite population estimates, which are not host destructive, a practical proposition. Further, the self-sustaining nature of the life cycle in laboratory conditions enables realistic simulations of natural infection processes to be achieved in an experimental situation.

MATERIALS AND METHODS

Parasitic maintenance

Infections of *Transversotrema patialense* in their snail host *Melanoides tuberculata*, and on their final host *Brachydanio rerio*, were maintained in the laboratory using the techniques and conditions which have been described previously (Anderson & Whitfield 1975). Maintenance of the infections and all population experiments were carried out in the temperature range 23–25° C. This range approximately corresponds with the ambient May temperature of an aquatic habitat in Penang, Malaysia, known to contain a natural population of *Transversotrema patialense* (Dr C. Betterton, personal communication).

Cercarial production

Eight randomly infected specimens of *Melanoides tuberculata* in the weight class 150–360 mg were individually assessed for their cercarial production over thirty-five days. Snails were maintained in a 12 : 12 h, dark : light regime at 24° C in separate 30 ml capacity bowls and daily fed an excess of dried boiled lettuce leaf and a proprietary fish food. The cercarial production of each snail was counted directly each 24 h after the larvae had been immobilized by formaldehyde fixation..

The effect of snail starvation on cercarial production

Four randomly infected specimens of *M. tuberculata* were kept in individual bowls as described in the section above. After fourteen days of normal feeding, all feeding was stopped for eleven weeks and then resumed. Throughout this altered dietary regime, individual daily counts of cercarial production were made.

Cercarial survival

Separate batches of freshly shed cercariae were aged for varying periods of up to 48 h in 500 ml dishes of tapwater at 24° C in diffuse light of 150 lux intensity. At the end of each aging period percentage survival was directly assessed. A dead cercaria was defined as one which displayed no spontaneous movement and from which no movement could be elicited by three light contacts with a stainless steel needle.

Cercarial infectivity: effect of aging

Uninfected specimens of *Brachydanio rerio* in the length class 20–30 mm were used to assess the effect of cercarial age on infectivity. Batches of ten freshly shed cercariae were aged for periods between 0·25 and 24 h in 10 ml of tapwater at 24° C with diffuse lighting of 150 lux intensity. For each aging period five such batches were placed, each with a single fish, in 30 ml of tapwater in the same conditions for 30 min. At the end of this

infection period, the replicate fish were removed and the numbers of parasites attached to them counted directly while the fish were maintained under anaesthesia in an MS. 222 (Sandoz) solution (1:10 000 w/v in tapwater).

Cercarial infectivity: effect of density

Densities of from 5–200 freshly shed cercariae/500 ml tapwater were used to assess the effect of cercarial density on infectivity. Single uninfected specimens of *B. rerio* in the size class 20–30 mm were added to such 500 ml volumes at 24° C in 150 lux diffuse lighting for 2 h. At the end of this period the fish were removed and the number of parasites attached to each fish assessed directly as described above. For each density at least three replicates were assessed.

Cercarial infectivity: the nature of the infection process

To assess the distribution of infection levels in repeated replicate infections, 122 such infections were carried out with fish in the 20–30 mm length class. In each infection, each fish was exposed for 30 min to five freshly shed cercariae of *Transversotrema patialense*. The exposures were carried out in separate dishes of 30 ml of tapwater at 25° C in diffuse light of 150 lux intensity. After exposure, the number of parasites that had attached were counted as described above.

Adult survival

Eight uninfected specimens of *Brachydanio rerio* in the length class 28–32 mm were exposed to approximately thirty-five freshly shed cercariae for 2 h. Such an infection procedure usually produced infections of between 14–18 juvenile worms per fish. These infections were counted directly, after anaesthetizing the fish, within 24 h of the termination of cercarial exposure, and worms in excess of a total of fourteen were removed by use of a stainless steel microhook. Fish infected in this manner were maintained individually in 1 litre capacity tanks filled with tapwater at 23° C and subjected to aeration for 12 h/day and a 12:12 h, dark/light regime. The fish were fed daily on proprietary fish food. At least four times in each successive seven day period, the adult worm population was counted on each of the eight replicate fish while under anaesthesia. Counts were continued on each fish until three consecutive counts indicated no worms were present.

Immigration death experiment

Ten uninfected specimens of *B. rerio* in the length class 20–30 mm were subjected to consecutive weekly infections with freshly shed *Transversotrema patialense* cercariae for a total of nineteen weeks. The individually fin-clipped fish were maintained together in a 13 l tank with constant aeration and normal daily feeding. Each week each fish was individually exposed for 30 min to five cercariae. The exposures were carried out in dishes containing 30 ml of tapwater at 25° C in diffuse light of 150 lux intensity. The adult parasite population size on each fish was monitored under anaesthesia before and after each weekly infection. These pre- and post- infection counts enabled both the total parasite population on each fish and the number of new individuals that had entered the adult population at each infection to be continually assessed. Between the pre-infection count and the infection itself, fish were allowed to recover from their anaesthesia for 1 h in fresh tapwater at 25° C containing an excess of proprietary fish food. This food presentation constituted an attempt to satiate the fish to produce a relatively low and constant rate of cercarial predation by the fish (see Anderson & Whitfield 1975).

RESULTS

Dynamics of the free-living cercarial population

The size of the free-living cercarial population, a larval stage which does not reproduce, is simply controlled by two population processes, the immigration and death rates. Immigration is determined by the number of infected snails in the intermediate host population and the rate at which they release cercariae into the aquatic habitat. The death rate is a more complex process since it consists of three distinct components; natural mortalities, the rate at which larval parasites infect the final host to become adult parasites (emigration) and the rate of predation of the larvae by the fish host and other predators. This latter component is of considerable biological interest, the free-living cercarial stage being an attractive food source for small cyprinid fish. The rate of infection of the final host is thus highly dependent on the predator-prey interaction between definitive host and parasite.

The basic features of the dynamics of the cercarial population can be described by an immigration-death process. For simplicity, if it is initially assumed that the immigration rate λ is constant and independent of larval population size, and the death rate μ is also constant and simply proportional to the number of cercariae, then the following differential equation describes the rate of change in C_t, the number of cercariae at time t.

$$\frac{dC_t}{dt} = \lambda - \mu C_t. \qquad (1)$$

If there are C_0 larvae in the habitat at time $t = 0$, equation (1) has the solution

$$C_t = \frac{\lambda}{\mu}[1 - \exp(-\mu t)] + C_0 \exp(-\mu t). \qquad (2)$$

This model predicts growth to an equilibrium population size $\hat{C} = \lambda/\mu$ a state which is globally stable. In reality the immigration and death rates may not be constants but functions of other variables such as the time-dependent age and size structure of the infected snail population, the age of the larvae, or physical parameters such as water temperature. The simple model described above, however, provides a framework for the incorporation of more realistic biological assumptions. The expansion of this framework can best be considered in the light of a detailed knowledge of the immigration and death rates of the cercarial population.

Immigration

The rate of cercarial production by an individual snail will be dependent on a number of biological and physical parameters which can be considered as falling into three broad categories. The first of which concerns host mediated factors such as the size of the snail (Raisyte 1968; Wright 1971), its nutritional status (Kendall 1949; Coles 1973) and host reaction against parasitic infection such as encapsulation (Pan 1965). The second category includes parasite mediated factors such as the number of miracidia which successfully infect a snail, the age of the infection and the rate of asexual reproduction (Wilson & Draskau 1976). The final group consists of physical environmental factors such as temperature, pH of the habitat and the nature of daylight patterns (Rees 1948; Campbell 1973; Nice & Wilson 1974). All three categories are to some extent interrelated since, for example, environmental variables will influence the nutritional status of a

mollusc. In laboratory investigations of the population biology of a digenean parasite, in which physical variables such as temperature and day-night regimes are constant, the first two categories are of major importance.

Due to technical problems, it has not as yet proved possible to infect *Melanoides tuberculata* with single miracidia of *Transversotrema patialense*. In order to assess cercarial production rates it was found necessary to utilize infected snails collected from parasite maintenance tanks. The origin of the larval parasite population in these experimental animals, in terms of the number of miracidia which have successfully infected each snail and the age of the larval infections, was thus unknown.

Infected snails of a given size (weight) range (150–360 mg), showed a remarkable degree of constancy in their daily cercarial production rates. There was no significant degree of

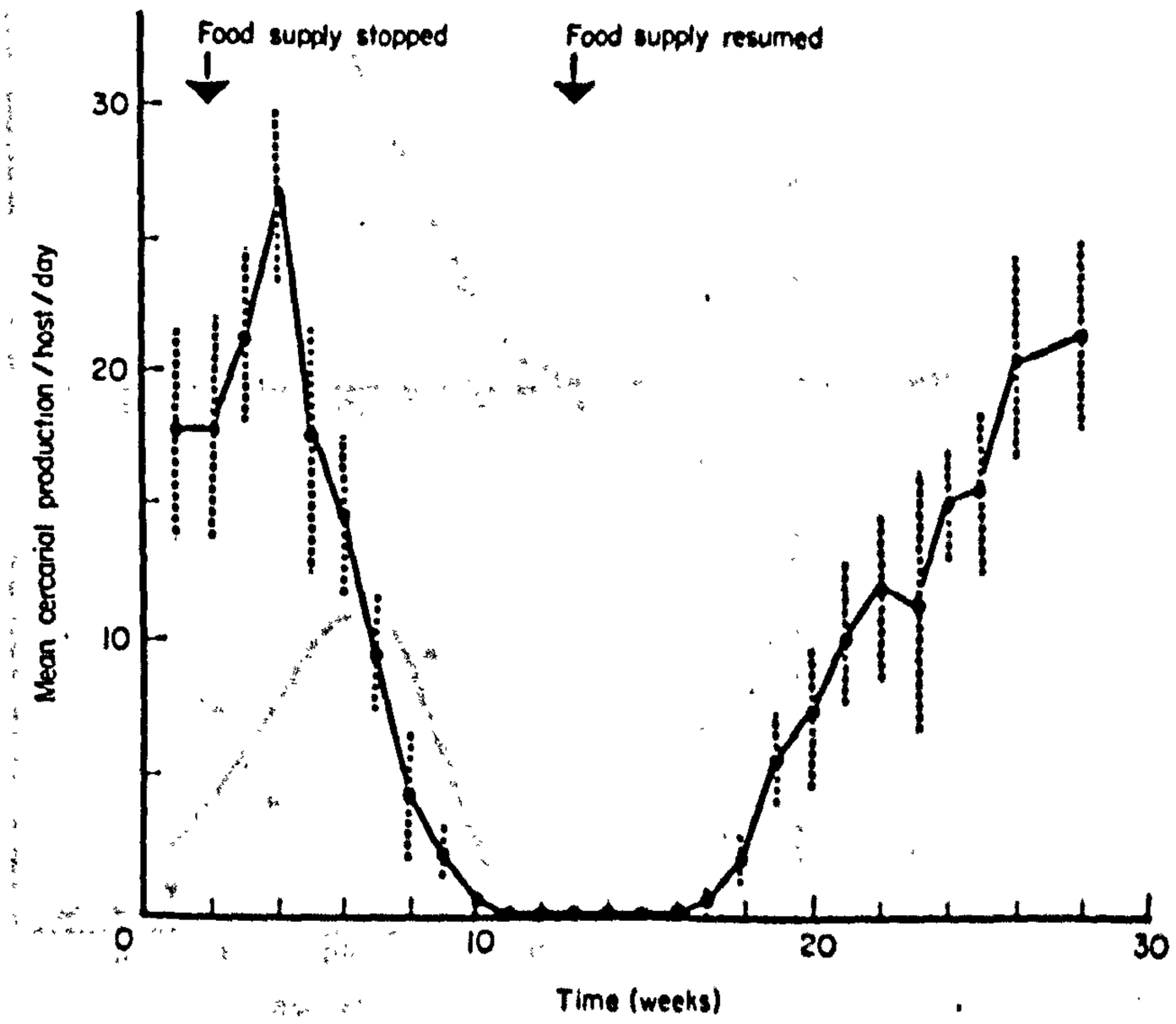

FIG. 2. The influence of host starvation on daily cercarial production by *Melanoides tuberculata*. Solid circles —mean daily cercarial production rates, estimated over one week periods for four infected snails; dashed vertical bars—95% confidence limits of the means.

correlation between weight of host and daily cercarial output rate (P ($r = 0{\cdot}147$) $> 0{\cdot}10$, d.f. = 6) amongst the infected snails used in these experiments. This result is to some extent surprising since the carrying capacity of the larval parasite's microenvironment is to a large extent determined by the size of the snail's digestive gland. Heavier snails should thus be capable of supporting higher production rates of cercariae (Wright 1971; Eveland & Ritchie 1972). The lack of significant correlation may be due to both the small sample size and more importantly the limited size range of hosts examined. In contrast, to these observations, preliminary results from a much larger weight range of infected *Melanoides tuberculata*, collected near Penang in Malaysia, indicate a significant positive

correlation between snail size and cercarial production rate (Dr C. Betterton, personal communication).

The daily cercarial outputs of the eight experimental snails showed no significant temporal trends over a thirty-five day monitoring period. Linear regression analysis of each snail's daily production (y) versus time (x), indicated that the slope of the best fit regression line was not significantly different from zero for each experimental mollusc.

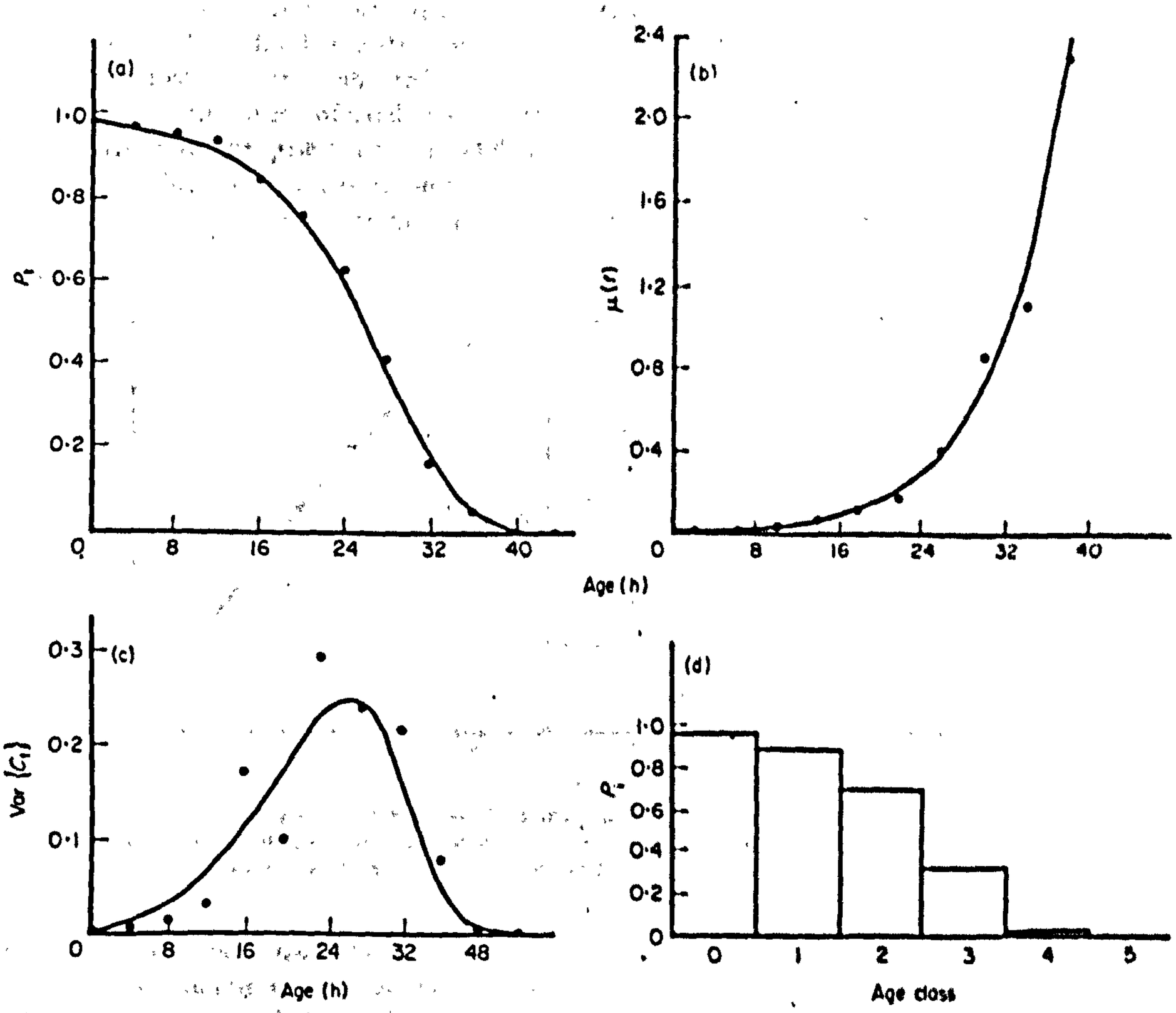

FIG. 3. Survival characteristics of the cercariae at 24° C; (a) proportion of larvae surviving at consecutive time points: solid circles—observed mean proportions (estimated from replicate experiments): solid line—expected proportions predicted by age-dependent survival model (equation (6)); (b) age dependency of the instantaneous death rate $\mu(t)$ / 4 h-period / larvae: solid circles–observed points: solid line—best fit exponential model of the form $\mu(t) = a \exp(bt)$, where a = 0·0082 and b = 0·5954; (c) the observed (solid circles) and expected (solid line) (equation (7)) variances of the proportion of cercariae surviving at various ages; (d) the age-dependent survival probabilities (P_i's) for five eight-h age classes of the larvae estimated from the predictions of the continuous time model (equation (6)).

In addition, time series analysis, involving the calculation of serial correlation coefficients for cercarial production on day t and day $t+x$, showed no significant correlations for values of x from one to eight days for each snail (Yule & Kendall 1965). It is thus reasonable to assume that the daily cercarial output rates form a random sequence in

time for each of the infected snails over the thirty-five day observation period. Longer time sequences of production rates for four snails over 130 days exhibit similar random patterns.

The nutritional status of a mollusc infected with a digenean parasite is known to have a marked influence on the rate at which cercariae are produced (Coles 1973). *M. tuberculata* infected with *Transversotrema patialense* is no exception, since the type and quantity of food provided to the intermediate hosts has a marked influence on larval parasite production. Although *Melanoides tuberculata* is capable of surviving for comparatively long periods without food, the development of the larval parasite within the digestive gland of the host is severely affected.

Laboratory maintained infected snails were normally provided with excess quantities of dried lettuce and proprietary fish food flakes. Figure 2 demonstrates the effect of host starvation on the rate of cercarial output for a group of four snails. The mean number of cercariae produced per day per host dropped to zero approximately nine weeks after

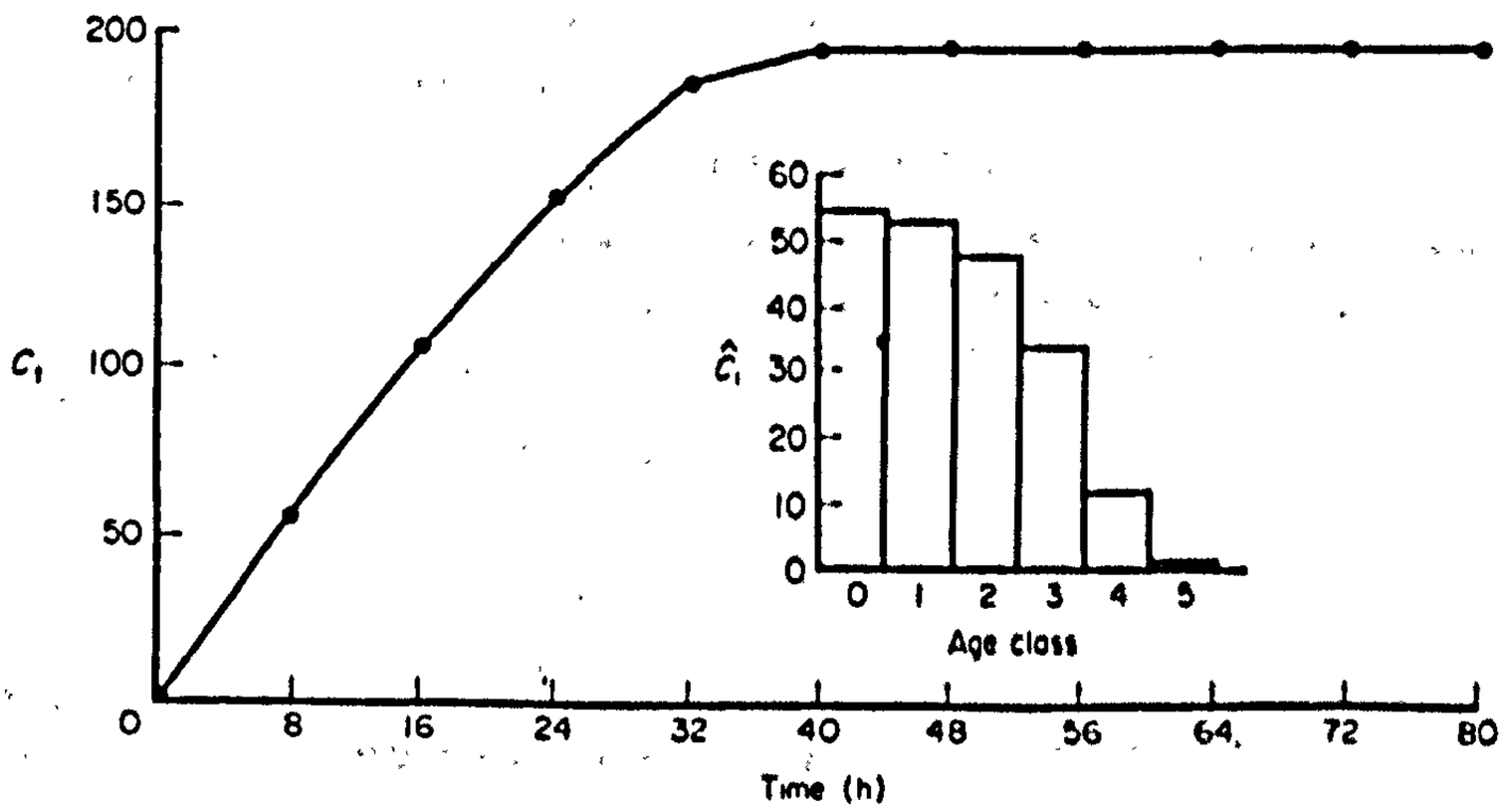

FIG. 4. The predicted growth of a cercarial population (C_t) subject to a constant immigration rate of 54·5 larvae per 8-h time period (equation (10)); the inset histogram shows the stable age distribution of the larval population at equilibrium.

the hosts' food supply had ceased. It is interesting to note, however, that the larval parasites were capable of continuing development and asexual reproduction once the food supply was resumed. A time lag of approximately 3·5 weeks elapsed before cercarial production restarted, although a lag of 8 weeks occurred before cercarial production rates regained their original levels.

The influence of multiple miracidial infections on cercarial output rates of individual snails has not as yet been determined due to the technical problems mentioned previously. It appears likely, however, that multiple infections of the snail intermediate host of digenean parasites do not in general lead to markedly increased cercarial production rates (Wilson & Draskau 1976). The constancy of daily output rates of the experimental snails of unknown infection origin provides some support for this suggestion in the case of *Transversotrema patialense*. It is likely that a single miracidial infection is capable of producing sufficient sporocyst or redial forms, due to the enormous asexual reproductive potential of larval digeneans, to saturate the carrying capacity of the parasite's micro-

environment within the molluscan host. The snails ability to absorb multiple miracidial infections without leading to markedly increased cercarial production may act as a major regulatory factor in the population dynamics of digeneans. In the case of *T. patialense* the existence of this form of density dependent mechanism requires experimental verification.

Survival of the cercarial stage

The cercariae of *T. patialense* have a maximum life span of approximately 44 h in tapwater maintained at a temperature of 24° C in a light intensity of 150 lux. The observed survival characteristics of populations of cercariae maintained in 500 ml dishes are illustrated in Fig. 3. The points of the graph represent the mean proportions of the initial cercarial population alive at time t, estimated from eight replicate experiments. The survival process is markedly age-dependent and the graph of μ, the instantaneous death rate per cercariae per 4 h time period, versus the age of the larvae illustrates the functional form of this dependency (Fig. 3(b)). The relationship can be empirically described by an exponential model of the form.

$$\mu(t) = a\exp(bt). \tag{3}$$

The constants a and b were estimated from the observed data by a non-linear least squares technique using Marquardt's algorithm (Conway, Glass & Wilcox 1970) and the fit of the model is illustrated in Fig. 3(b). The survival characteristics of a cercarial population subject solely to natural mortalities can thus be described by an age and thus time dependent model of the form

$$\frac{dC_t}{dt} = -\mu(t)C_t \tag{4}$$

where C_t is the number of larvae surviving at time t. Given that there are C_0 larvae at time $t = 0$, and the exponential form of $\mu(t)$, equation (4) has the solution.

$$C_t = C_0\exp\left[\frac{a}{b}[1-\exp(bt)]\right]. \tag{5}$$

The analogous stochastic model of this process has been described by Anderson & Whitfield (1975) and predicts a positive binomial distribution for the probability $P_c(t)$ of observing c cercariae alive at time t. The mean and variance of this distribution are,

$$\mathrm{E}\{C_t\} = C_0\exp\left[-\int_0^t \mu(v)\,dv\right] \tag{6}$$

and

$$\mathrm{Var}\{C_t\} = C_0\exp\left[-\int_0^t \mu(v)\,dv\right]\left[1-\exp\left(-\int_0^t \mu(v)\,dv\right)\right]. \tag{7}$$

The observed means and variances of the number of cercariae surviving at various points in time show good agreement with the predictions of the model (Fig. 3(a), (c)). The mean expected life span of a cercaria of age 0, estimated from the exponential model of the instantaneous death rate was 25·35 h at 24° C.

The brevity of the larval life span is undoubtedly associated with the fact that the cercariae of digeneans are in general actively mobile and probably non-feeding, possessing a finite nutrient reserve. In the case of *T. patialense* an important component of this reserve is glycogen (Anderson & Whitfield 1975). The progressively diminishing energy

reserves of the cercariae almost certainly generate the age-dependent death rate illustrated in Fig. 3(b).

A convenient way of representing the continuous time age-dependent death process of the larval parasites is to partition the maximum life span into a series of age classes, the members of which have various probabilities of surviving to the next age class. A survival probability P_l can thus be defined as the proportion of individuals of age l time units which survive to age $(l+1)$. The age-dependent survival probabilities for the cercariae of *T. patialense*, estimated from the predictions of the continuous time model (equation 5) are illustrated in the form of a histogram in Fig. 3(d), for six 8-hourly age classes.

Immigration-death model of the cercarial population with age-dependent survival rates

The immigration-death model of the dynamics of the cercarial population described earlier (equation 1) contained the assumption that both the immigration and death rates λ and μ, were constant.

In the laboratory, within an experimental tank containing a constant number of infected snails, the daily immigration rate of cercariae into the free-living population can reasonably be assumed to be approximately constant. The temporal constancy of the output having already been demonstrated. For the eight snails used in obtaining the daily production rates, the average daily rate per snail was 20·43 larvae (95% confidence limit ±5·25). The average total output of cercariae into an experimenta tank containing all eight snails is thus 54·48 per 8-h time period.

The death rate, however, cannot be assumed to be constant due to the age-dependency of the larval survival characteristics (Fig. 3). In the case of a cercarial population in which deaths are entirely due to natural mortalities, the incorporation of the age-dependent rates into the immigration-death model can be facilitated by changing from a continuous to a discrete time formulation. If C_t is a column vector containing the elements $c_l(t)$ denoting the number of cercariae in the age class l at time t, then the appropriate model is of the form

$$C_{t+1} = C_t \Lambda + \Lambda \tag{8}$$

where one unit of time is equivalent to 8 h. The maximum age class reached by the cercariae of *T. patialense* is the sixth 8-h class and thus the vector C_t contains six elements where $l = 0, 1, \ldots 5$. The matrix Λ is a transition matrix, containing the survival probabilities, the P_l's on the off diagonal.

$$\mathbf{A} = \begin{pmatrix} 0 & 0 & - & - & 0 \\ P_0 & & & & - \\ 0 & P_1 & & & - \\ - & & - & & - \\ - & & & - & - \\ 0 & - & - & P_4 & 0 \end{pmatrix} \tag{9}$$

This transition matrix is in essence a Leslie matrix in which the age-dependent reproductive rates are all zero since the cercarial population does not reproduce. The column vector Λ is the immigration vector of order six, where the only non zero element is λ_0, since all the cercariae released from the infected snails enter the zero cercarial age class.

The general solution of equation (6) is—

$$C_t = \Lambda' C_0 + (I-\Lambda)^{-1}(I-\Lambda')\Lambda \qquad (10)$$

where C_0 is the vector of the number of cercariae in each age class at time $t = 0$ and I is the unit identity matrix. When the time parameter t becomes greater than the maximum life span of the larval parasite, an equilibrium population size $\check{C}$ is achieved where

$$\check{C} = (I-\Lambda)^{-1}\Lambda. \qquad (11)$$

A stable age distribution exists at this equilibrium state, the proportion of the larvae in each age class being directly estimateable from the age-dependent death function (equation (3)). The growth of a population of cercariae, as predicted by equation (11), and subject to constant immigration generated by the cercarial output from the eight infected snails used in the larval release experiments described earlier and natural mortalities of the form depicted in Fig. 3, is shown in Fig. 4. A stable age distribution is reached after approximately 48 h. At equilibrium, the death rate of the population as a whole remains constant. Although each age class has its own age-specific death rate, the proportions in which the age classes are present remain constant.

In reality the death rate is of a more complex form than the process incorporated in the immigration-death model. As mentioned previously, two other distinct components influence the overall mortality rate; infection of the final fish host by the larvae resulting in emigration from the free-living cercarial population, and predation of the larvae by the fish host. The infection process leads to immigration into the adult parasite population and will be described in the following section of this paper.

Predation is a much more complex phenomenon, the rate of which depends on a variety of factors, the most important being the density of larvae in the habitat or experimental chamber. Preliminary results indicate that the relationship between the number of cercariae eaten by *Brachydanio rerio* and the density of larvae is of sigmoid form where the number eaten approaches an upper asymptote at high cercarial densities, however, the rate of predation is an increasing function of larval parasite or prey density and this the functional response of the fish predator can be classified as a type III response in the terminology of Holling (1959, 1961, 1965). The fish appears to spend a progressively increasing proportion of its time feeding on the prey as the density of cercariae rises. The rise to an upper asymptote is due to both the handling time of the prey by the predator and the maximum ration of food the fish can consume in a unit period of time (Hassell, Lawton & Beddington 1976; Ivlev 1961). The nature of this functional response, however, is dependent in experimental conditions on a number of other factors such as volume of experimental chamber, density of alternative food sources and temperature of environment. Detailed experimental results concerning the form of this functional response will be described in a further publication. In this present paper the dynamics of the cercarial population will be considered in isolation from the predatory activities of the fish host.

Dynamics of the adult parasite population on the fish host

As in the case of the cercariae, an adult parasite population on a single host, is simply controlled by two processes, the immigration and death rates. Immigration is determined by the rate of infection of an individual fish by the cercariae and thus is primarily dependent on cercarial density in the habitat. In contrast to the free-living cercarial stage, the death rate of the adult parasite consists of a single component created by natural mortalities on the fish host. Such mortality may be due to senescence, immune responses or density dependent effects

If both immigration and death occur at constant rates, a model of the form described in equation(1) will describe the essential features of the dynamics of the adult parasite on a single host. In practice both rates are unlikely to be constant and hence a detailed knowledge of their relationship with other variables is ideally required.

Immigration or Infection

In environmental conditions of constant temperature, fish density and dark-light regimes, the infection process is primarily controlled by two factors, the age structure of the cercarial population and the density of larvae per unit volume of water.

Although the survival characteristics of the cercariae are of obvious importance in the

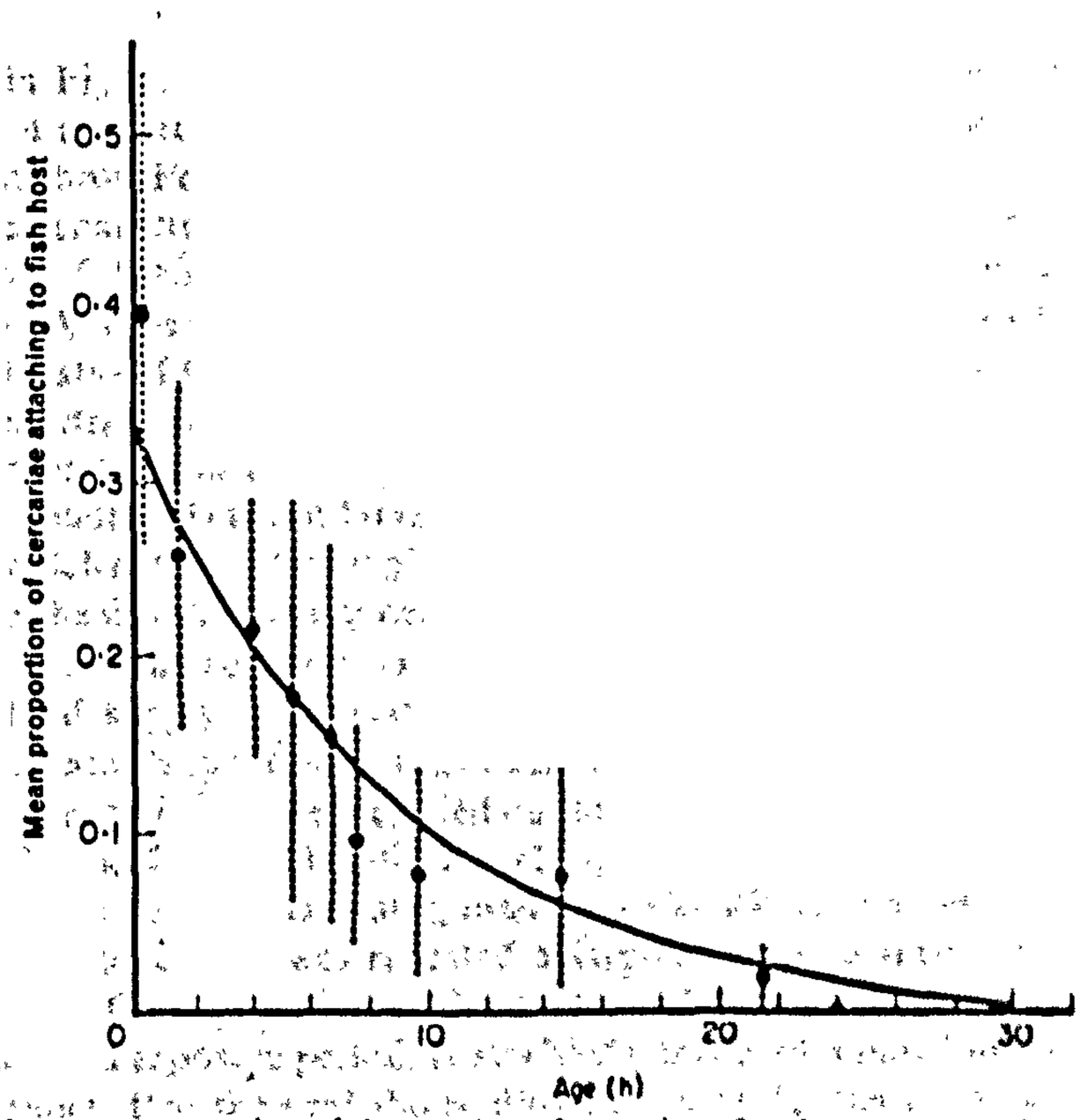

FIG. 5. The mean proportion of the number of cercariae of a given age to which a host is exposed, which attach to the fish in a unit period of time (30 min) (solid circles); solid line—best fit exponential of the form $g(t) = x \exp(-yt)$, where $x = 0{\cdot}332$ and $y = 0{\cdot}114$.

dynamics of the adult parasite population, they are unlikely to be directly associated with the ability of the larvae to successfully infect the fish host. A larval parasite which has survived for a period of say 40 h, may not have sufficient energy reserves left to enable the infection process to be completed successfully. The attachment process is an active one requiring a series of behavioural responses which necessitate specific and co-ordinated neuromuscular activities (Whitfield, Anderson & Moloney 1975; Whitfield, Anderson & Bundy 1976). Because of the finite energy reserves of the essentially non-feeding larvae, infection is likely to be an age-dependent process. A series of experiments were carried out to examine this possibility, in which fish which had previously been provided with an excess of food, were exposed to a constant density of larvae of known age for a short and

standardized exposure period. The proportion of larvae of known age, which managed to successfully infect the host and establish under the scales of a fish during this period is shown in Fig. 5. As expected, infectivity decreased rapidly as the cercariae aged and larvae older than 22 h failed to establish on the fish. This time span is of similar duration to the period during which spontaneous swimming activity of the larvae occurs (Anderson & Whitfield 1975). Swimming activity is undoubtedly a prerequisite for successful infection. A number of other workers have also recorded age-dependency in larval digenean infectivity (Evans & Stirewalt 1951; Miller & McCoy 1930; Olivier 1966). The relationship between the observed proportion of the density of the larvae to which the fish was exposed which attached to the host, g, and the age of the cercariae t, can be empirically described by an exponential model of the form

$$g(t) = x\exp(-yt) \tag{12}$$

as shown in Fig. 5. A further factor which may be of importance in determining the infectivity of the cercariae, relates to the experience of the developing larvae in the snail intermediate host. For example, the nutritional status of a host may determine not only the survival characteristics of the larvae, but also its ability to infect the final host. In the case of *Schistosoma mansoni* (Sambon) cercariae developing in *Biomphalaria glabrata* (Say), however, Eveland & Ritchie (1972) have demonstrated that although the nutritional status of the snail influences the number of larvae produced, it does not affect cercarial infectivity potential.

Infection of the fish host appears to be essentially a chance process, dependent on random encounters between larvae and fish. No specific behavioural responses of the larvae, in which directed 'homing' activities are indicated have as yet been demonstrated. In addition the fish does not appear to exhibit avoidance behaviour when in the vicinity of the cercariae. The converse, in fact, is true since fish actively seek out the larvae as a potential food supply. The relationship between cercarial density and proportion of larvae which attach to a host is thus likely to be one of direct proportionality.

A series of experiments were carried out to examine the nature of the infection process. Individual hosts of a given length class (26–32 mm) were exposed for a period of 30 min to five larvae in 30 ml of tapwater, maintained at 24° C in a light intensity of 150 lux. This infection procedure was repeated a large number of times with different fish, in order to assess the distribution of the number of parasites which attach per fish during the standardized exposure period. A stochastic model of a pure immigration or infection process predicts that the probability distribution of P_n, the probability of observing n parasites on a given host, is of Poisson form. In the case of experimental procedure used in this present study, however, the positive binomial is a more appropriate distribution. This limiting form of the Poisson model is generated by the high infection probability (p) and the finite limit to the number of parasites that can attach to a single host during the 30 min exposure period. These constraints are entirely due to experimental design.

If infection is essentially a change process for a fish of given size class, the P_n should be of the form

$$P_n = \binom{5}{n} p^n q^{5-n}.$$

The observed frequency distribution of the number of immigrant parasites per host showed good agreement with the predictions of the positive binomial model ($P(\chi^2 = 4{\cdot}78) > 0{\cdot}05$, d.f. = 5).

Additional experiments were carried out to examine the relationship between exposure density of cercariae and the number which attach to a host. Fish were exposed in experimental containers of constant size (500 ml) to varying densities of larvae 5–200 larvae/500 ml for a short and standardized exposure period. The observed relationship between the mean proportion of larvae which managed to attach per fish and the density of larvae is

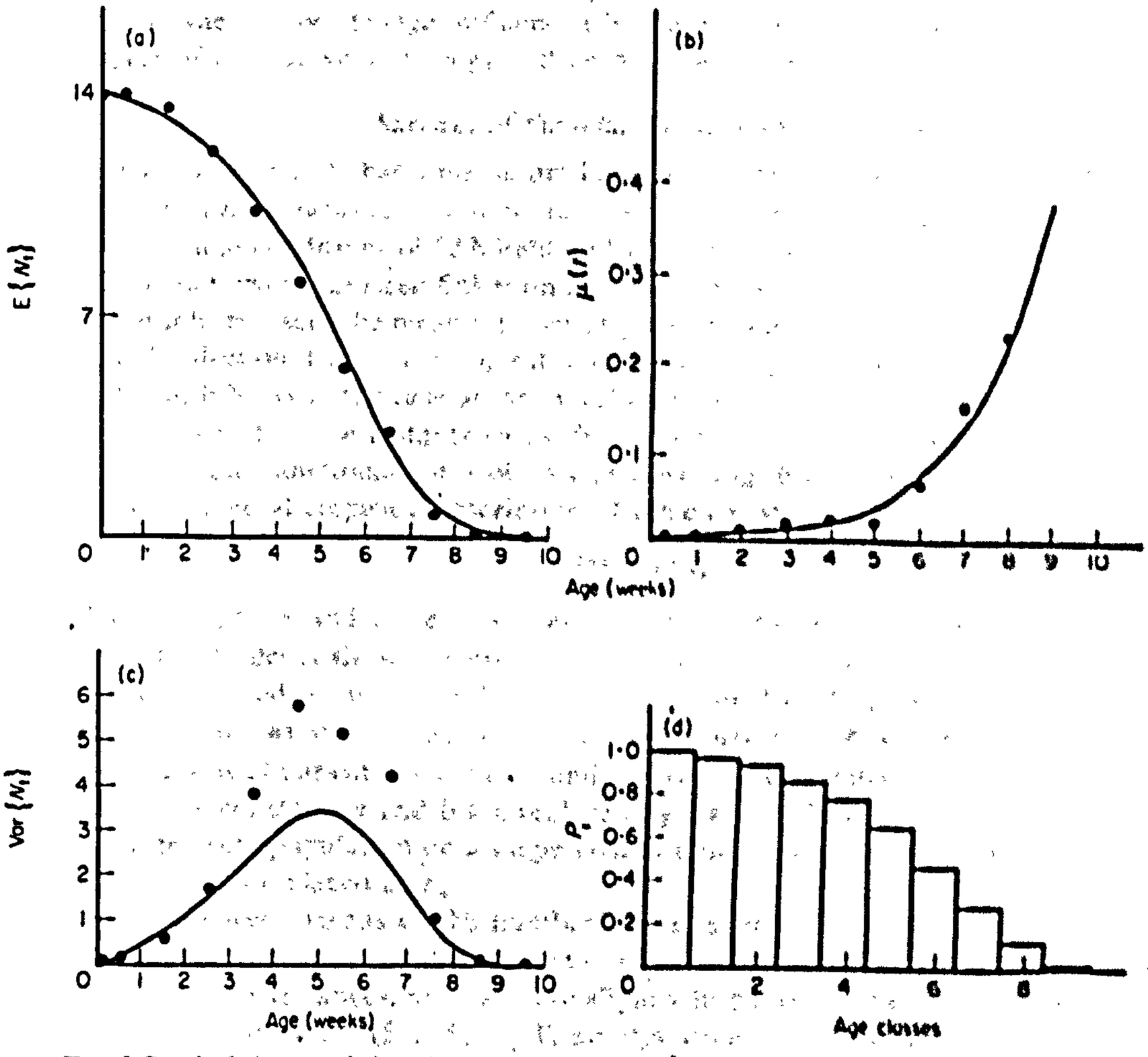

FIG. 6. Survival characteristics of the adult parasite on the surface of the fish host at 24° C; (a) mean number of parasites surviving at various points in time: solid circles—observed means, solid line—expected numbers predicted by the age-dependent survival model (equation (6)); (b) age-dependency of the instantaneous death rate $\mu(t)$ / week / host: solid circles—observed points, solid line—best fit exponential model of the form $\mu(t) = \alpha \exp(\beta t)$, where $\alpha = 0{\cdot}0242$ and $\beta = 0{\cdot}5591$; (c) the observed (solid circles) and expected (solid line) (equation (7)) variances of the number of parasites surviving at a series of consecutive points in time; (d) the age-dependent survival probabilities (P_x's for ten weekly age classes of the adult parasite, estimated from the predictions of the continuous time model (equation (6)).

of linear form and the least square best regression line, constrained to go through the origin, has a slope of 0·232 ($P(r = 0{\cdot}972) < 0{\cdot}001$, d.f. = 18).

It is important to note that a further biological component operates in these experiments, the predation of the larvae by the fish host. The predation rate, as mentioned eariler, is not simply proportional to larval density. In contrast the instantaneous rate of

infection is a constant, the number of larvae establishing on the fish being directly proportional to the available density of cercariae. Interference between larvae, with respect to attachment efficiency has not been demonstrated for densities of larvae up to 1·0 cercariae/ml of water. Furthermore, the rate of attachment has been shown to be independent of the number of parasites already established on a given host for densities up to forty parasites/host (C. A. Mills, unpublished results).

In any long term experiment of the parasite population dynamics the density of available larvae will be strongly influenced by the predatory activities of the fish host. This aspect of the parasites' biology will be discussed more fully in a further publication.

Survival of the adult parasite on the fish host

The adult parasite has a maximum life span of approximately 10 weeks on the surface of *Brachydanio rerio* (26–32 mm size class), maintained in tapwater at approximately 24° C and in conditions of 12 h light and 12 h dark. The observed survival characteristics of populations of fourteen flukes on single fish are illustrated in Fig. 6(a). The points on the graph represent the mean number of parasites surviving at half weekly intervals from initial infection (time, $t = 0$), estimated from eight separate experiments involving different fish. As in the case of the larval parasites, the survival process is age-dependent and a plot of μ, the instantaneous death rate per adult parasite per week *versus* age illustrate the functional form of this process (Fig. 6(b)). An exponential model again provides a good empirical description of the data where,

$$\mu(t) = \alpha \exp(\beta t). \tag{13}$$

The constants α and β were estimated as described previously (equation (3)) and the fit of the model is shown in Fig. 6(b). The survival characteristics of the adult parasite can be depicted by an age and thus time-dependent death process of the form outlined in equations (4) and (5). Similarly, the appropriate stochastic model, for small initial populations of parasites, has mean and variance as determined by equations (6) and (7), where the constants a and b are replaced by α and β. In addition if n_t is the size of the adult parasite population on a single host at time t, the constant C_0 in equations (4), (5), (6) and (7) is replaced by n_0.

The observed means of the number of parasites surviving at various points in time show good agreement with the predictions of the model (equation (6) and Fig. 6(a)). The observed variances, however, are slightly larger on average than those predicted by the stochastic model (Fig. 6(c)). These deviations from the predicted trend are most probably generated by variability in the suitability of a host surface as a microenvironment for the ectoparasite, resulting in heterogeneity in survival of fluke populations between hosts. Additional variability may also be created by between-fluke differences due to genetic determinants or other factors generated by the parasites' experiences in the snail intermediate host.

The degree of variability in survival characteristics of populations of parasites on different hosts is illustrated in Fig. 7, in which the histories of the populations on two fish are recorded. The mean expected life span of an adult parasite of age zero, estimated from the exponential form of the instantaneous death rate is 4·81 weeks. The continuous time, age-dependent survival function can also be used to calculate the survival probabilities (p_l) of surviving from age class l to $(l+1)$. These are tubulated in the form of a histogram in Fig. 6(d) for each of ten weekly classes.

The age-dependency of adult parasite survival may be generated by a variety of

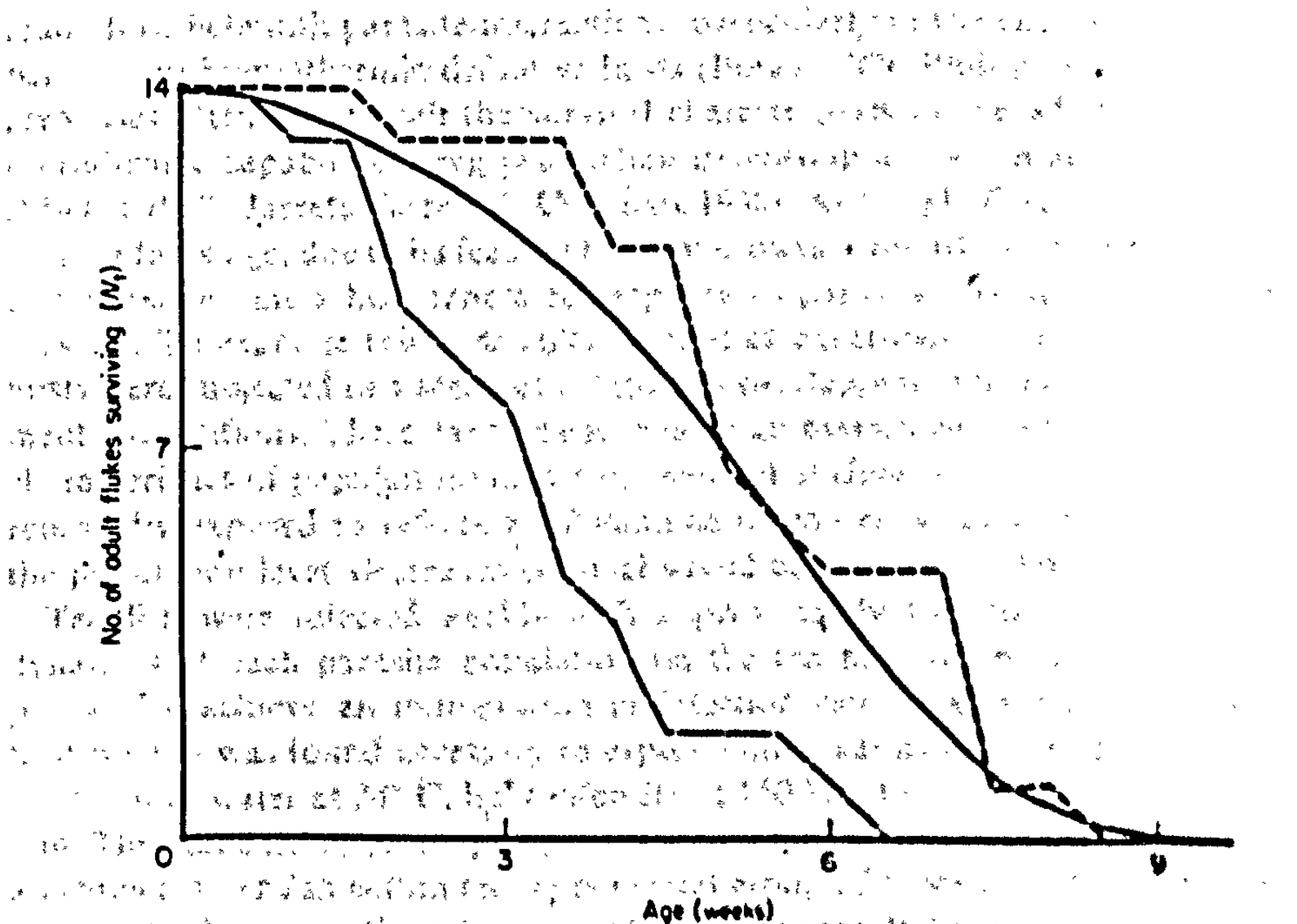

FIG. 7. Histories of two fourteen-fluke populations on separate fish (dashed lines) compared with the expected survival curve (solid line) (equation (6)).

biological mechanisms, the two most obvious of which are senescence and a time-dependent host generated immune response. This latter possibility will be discussed at a later stage in this paper.

Other factors such as water temperature and density of the adult parasite population also influence the survival characteristics of the fluke. Temperature is a particularly important environmental variable, affecting not only survival of larval and adult digeneans in poikilothermic hosts, but also the rate of larval development in the molluscan intermediate host and adult maturation in the definitive host (Nice & Wilson 1974; Ollerenshaw 1971). The relationship between adult and larval survival of *Transversotrema patialense* and water temperature will be explored in a further publication.

Density dependent survival of the adult flukes on the fish host, has not as yet been demonstrated for infections up to forty parasites/host. Intraspecific competition for available food supplies (the fluke browses on the mucous and epithelial cells of the fish skin) and, more importantly, space on the surface of the host will undoubtedly lead to increased mortality of parasites at high densities. Space is a particularly important resource, since a fish of given size possesses a finite number of scales, and the recesses under these provide the microenvironments for the adult parasites. Of this total number of scales, only a certain proportion are large enough to provide effective protection against dislodgement for *T. patialense*. Thus the carrying capacity of a fish of given size will be far less than the total number of scales.

Immigration—death experiments

Host generated immune responses against parasitic invasion are known to occur in

many host-helminth parasite interactions, particularly in the case of adult worms endoparasitic in homiothermic definitive hosts (Boray 1969, Phillips *et al.* 1975). Such host responses often affect both the survival characteristics of the adult parasites and their reproductive capabilities; egg production decreasing as the immune response develops (Michel 1967; Jarrett, Jarrett & Urquhart 1968). Although *T. pattalense* is ectoparasitic in the adult stage, due to its feeding habit of browsing on fish mucous and epithelial cells, it is possible that a host-generated response to parasitic invasion may influence fluke survival. To examine this possibility a series of experiments were carried out in which hosts were subjected to a series of temporally overlapping infections in constant environmental conditions. These experiments enable an assessment to be made of the survival characteristics of populations of parasites on the surface of fish which have already been repeatedly exposed to infection. If immune responses occur and are of importance at the population level, decreased survival would be expected in later infections.

Ten fish were infected weekly with approximately two parasites per host and the dynamics of each parasite population on the ten fish was monitored over a 19 week period. To achieve an immigration or infection rate of approximately two parasites/host/week it was found necessary to expose individual fish in the 26–32 mm length class (in 30 ml of water at 24° C, light intensity of 150 lux) to five cercariae for a period of 30 min. This standard infection procedure led to some variability in the number of successful attachments per fish within an experimental group of hosts of similar size. As discussed previously, however, the infection procedure is essentially a chance phenomenon and the frequency distribution of the number of parasites per host generated by experimental infection methods is empirically described by the positive binomial model.

It is possible to place a known number of parasites on the surface of an anaesthetized host. By this method, it is theoretically possible to create a constant immigration rate per unit of time into the adult parasite population on the experimental fish. This technique however, places considerable strain on the fish due to the comparatively long periods during which the host has to remain under the anaesthetic. For this reason, the chance infection procedure was utilized in the immigration-death experiments even though it led to variability in the mean number of immigrants received per fish by the experimental group of hosts during the course of the nineteen week population experiment. The weekly fluctuations in the mean immigration rate showed no obvious temporal trends and the slope of the linear regression of mean immigration rate versus time was not significantly different from zero ($P(t = 0{\cdot}221) > 0{\cdot}05$, d.f. = 17). The overall mean of the immigration rate per fish per week, estimated from the week experimental period was 2·14 flukes/fish with a variance of 1·48.

The chance infection procedure used in these experiments had an additional virtue in that it more closely approximates the mechanisms of infection that occur in non-laboratory populations of hosts when compared with constant infection rates. In fact a greater degree of variability in parasite numbers per host than was generated in these experiments would arise in natural habitats due to the many biological and physical parameters which create heterogeneity in parasite immigration between members of a host population (Anderson 1974, 1976b).

The observed mean numbers of parasites per host, recorded over the 19 week experimental period are shown in Fig. 8. The average fluke burden rises to a plateau after about ten weeks and then remains approximately constant for the remaining experimental period.

In order to assess any changes in survival, these observed results must be compared

with the predictions of a model describing the dynamics of a population subject to a constant immigration rate and an age-dependent survival process which does not alter in character over successive time periods.

Model of the population dynamics of the adult parasite on the fish host

In the case of parasite species such as *T. pattalense* which do not multiply within or on the definitive host, the numerical size of an adult parasite population is simply determined by the immigration and death rates. The immigration rate was approximately constant in the temporally overlapping experiments while the death rate of the fourteen fluke infections (Fig. 6(b)) was highly age-dependent. The appropriate model for such a system is thus the same as that described for the dynamics of the cercarial population (equation (8)).

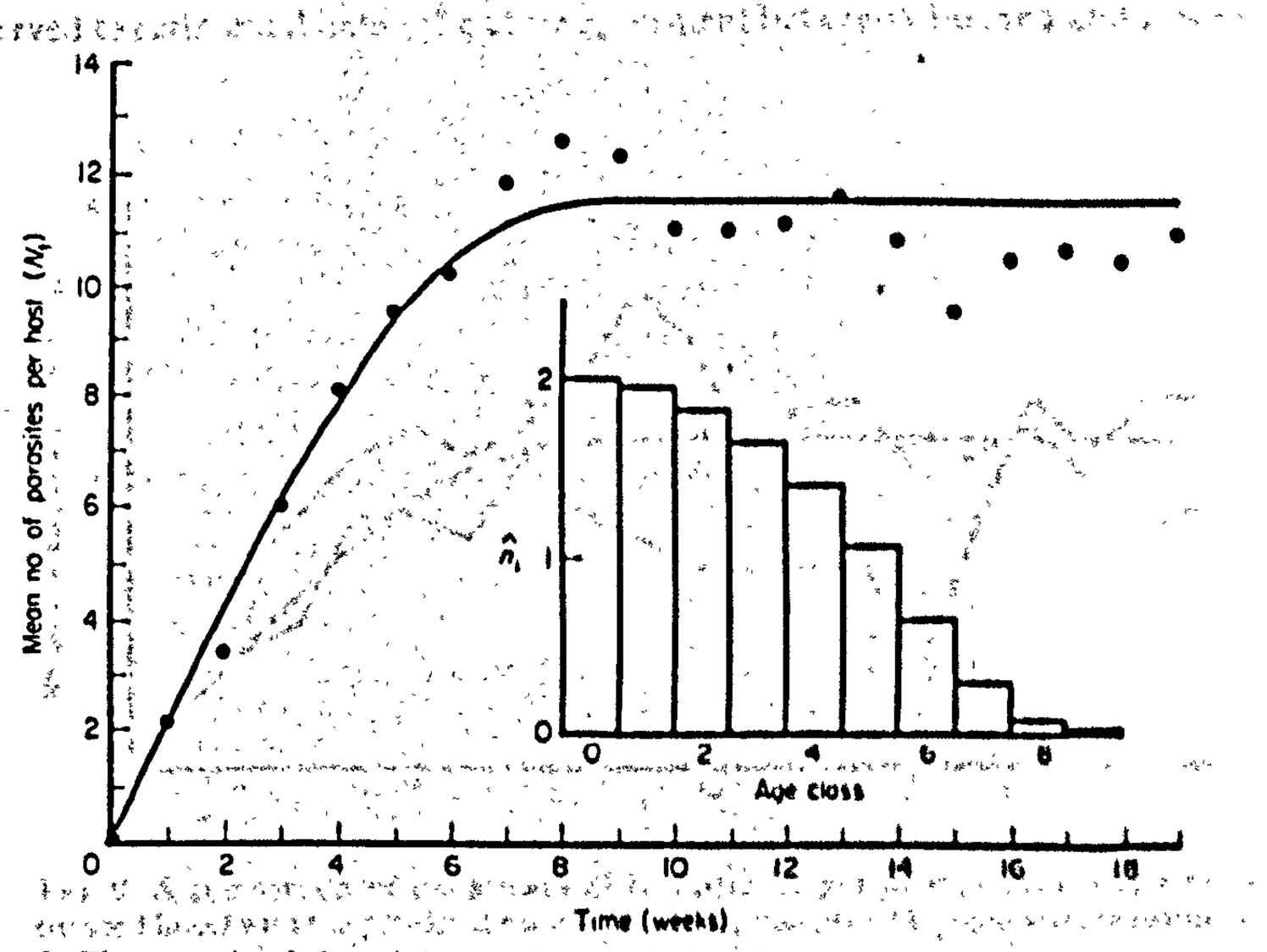

FIG. 8. The growth of the adult parasite population (denoted as the mean number of parasites/fish) subject to a constant weekly immigration rate of 2·14 parasites / host: Solid circles—observed points, solid line—predictions of the age-structured immigration-death model (equation (15)). The inset histogram shows the predicted stable age distribution of adult parasites at equilibrium.

If N_t is a column vector containing the elements $n_i(t)$ denoting the number of adult parasites on a single fish in the age class i at the time of t, then the appropriate model is of the form

$$N_{t+1} = N_t B + \gamma \qquad (14)$$

where one unit of time is equivalent to one week.

The maximum age class reached by the adult parasites as demonstrated by the survival experiments (Fig. 6(d)) is the 9-week-old age class and thus the vector N_t contains ten elements where $i = 0, 1, 2, \ldots, 9$. The matrix B is a ten by ten transition matrix of similar form to the matrix A (equation (9)), containing the survival probabilities, the

P_1's, on the off diagonal. γ is a column vector of order ten, determining the immigration rate, where the only non-zero element is γ_0, since all cercariae which attach to a host and enter the adult parasite population join the zero age class. For comparison with the observed results obtained in the immigration-death experiment, $\gamma_0 = 2{\cdot}14$ parasites/host/week, then the general solution of (14) is

$$\mathbf{N}_t = \mathbf{A}^t\mathbf{N}_0 + (\mathbf{I}-\mathbf{B})^{-1}(\mathbf{I}-\mathbf{B}^t)\boldsymbol{\gamma} \qquad (15)$$

with an equilibrium population size $\hat{\mathbf{N}}$ of

$$\hat{\mathbf{N}} = (\mathbf{I}-\mathbf{B})^{-1}\boldsymbol{\gamma}. \qquad (16)$$

The predictions of this model (equation (15)) employing the immigration rate of 2·14 parasites per week and the survival probabilities estimated from the adult fluke survival experiments (Fig. 6 (d)) are shown in Fig. 8. It can be seen that they closely mimic the observed experimental data. The close agreement between observed and predicted results

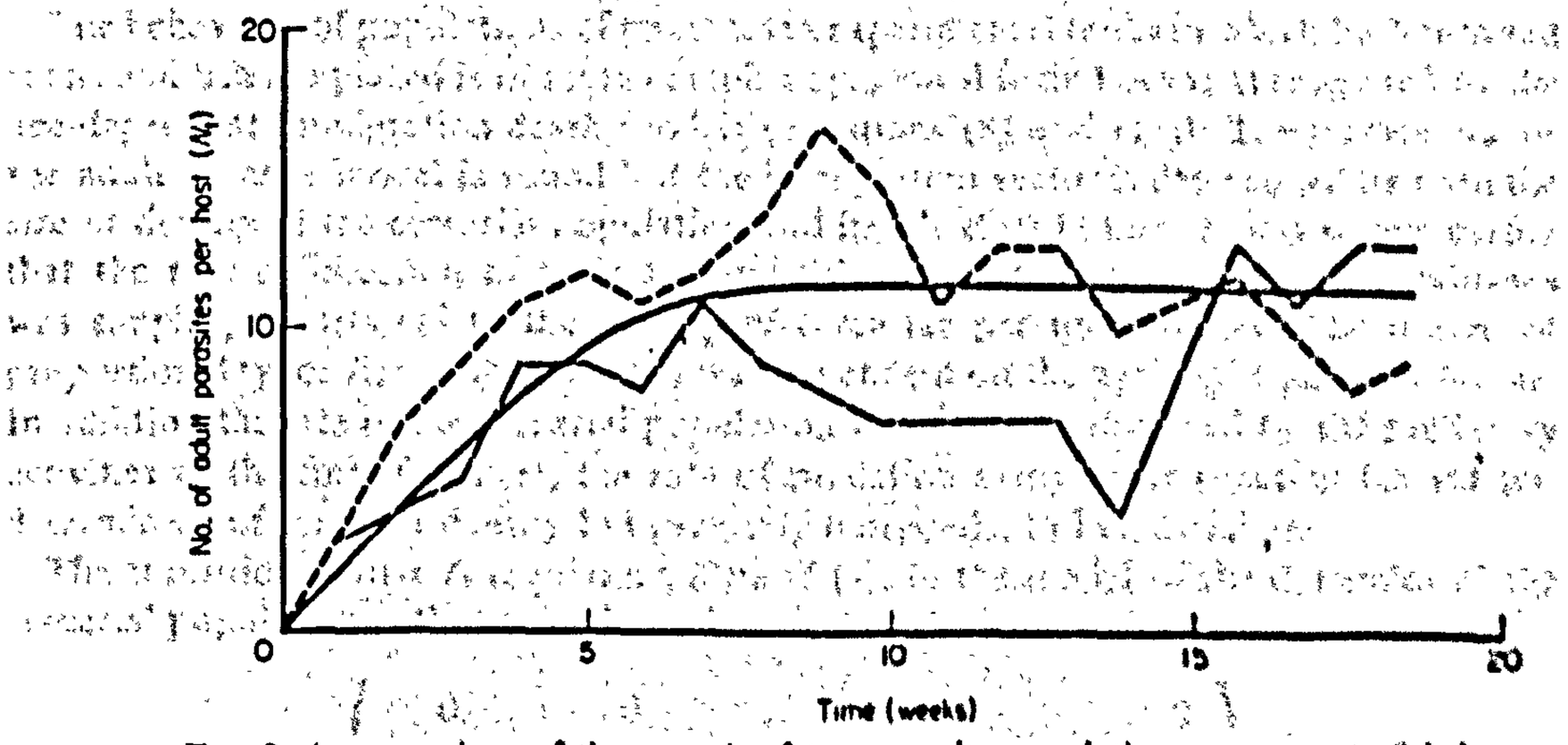

FIG. 9. A comparison of the growth of two parasite populations on separate fish hosts (dashed lines) with the predicted mean parasite burden per host (solid line) (equation (15)).

confirms the constancy of the survival characteristics of populations of flukes on fish which have been repeatedly exposed to low infections. It should be noted, however, that these results do not preclude the possibility of high infection levels, resulting in decreased worm survival.

Considerable variability exists in the parasite population sizes on individual fish at any given point in time. Fig. 9 shows the histories of two parasite populations on separate fish. This observed degree of variability is to be expected when populations are of small size, due to demographic stochasticity in both immigration and survival processes. The chance infection procedures, used in the experimental design created further heterogeneity both between fish and through time.

A stochastic model of an immigration-death process in which the death rate is age independent and the immigration rate is constant, predicts a Poisson distribution at equilibrium for the probability $P_n(t)$ of observing n parasites on a single host at time t

(Anderson 1974). If the immigration rate per fish at a given point in time is a random variable of Poisson form then a probability model of the population process predicts the Neyman type A distribution for $P_n(t)$ (Anderson 1976c). Heterogeneity in immigration thus creates over-dispersion in the counts of parasite numbers per host. A full stochastic model of an age-dependent immigration-death process is more difficult to formulate but equilibrium distributions have been examined by Seal (1945) and more recently by Pollard (1967). For a constant immigration vector, the number of parasites in each age class at equilibrium form a series of mutually independent Poisson variables and thus the distribution of the total parasite population size per host is itself Poisson. This result is identical to the age-independent immigration-death stochastic model. Similarly where the immigration vector contains elements which are themselves Poisson variants, the equilibrium distribution of the number of parasites in each age class are non-independent, positively correlated Poisson variates and thus the distribution of the total number of parasites per host is overdispersed.

The dynamics of both the cercarial and adult parasite populations

The behaviour of populations of parasites in experimental tanks in which both infected snails and fish are placed is of more complex dynamical form than that suggested by the age-dependent immigration-death models (equations (8) and (14)). The framework of the adult parasite model is sound but the immigration vector is determined by both the size or density of the cercarial population and its age distribution. It was shown earlier that the rate of infection of an individual fish in constant environmental conditions was simply proportional to the density of cercariae per unit volume. The degree of proportionality, or size of the constant was dependent on the age of the parasitic larvae. In addition the size of the cercarial population was further influenced by the predatory activities of the final fish host, the rate of predation being some function (as yet undetermined) of cercarial density but probably independent of cercarial age.

The transition matrix Λ (equations (8) and (9), in the model of the dynamics of the cercarial population is thus time dependent and of the form

$$\Lambda(t) = \begin{pmatrix} 0 & 0 & - & - & 0 \\ P_0(C_0(t)) & 0 & & & 0 \\ 0 & P_1(C_1(t)) & & & 0 \\ - & - & & & - \\ - & - & - & & - \\ 0 & 0 & & P_*(C_*(t)) & 0 \end{pmatrix}$$

where the transition probabilities are of the form.

$$P_i(C_i(t)) = P_i = F[g_i + q_i(C_i(t))].$$

The constant F represents the number of fish present in the habitat or experimental tank, while the terms g_i and $q_i(C_i(t))$ represent the proportional losses due to infection and predation respectively. The g_i terms are the age dependent infectivity constants estimated from equation (12) and the relationship between cercarial density and infectivity. The nature of the function $q_i(C_i(t))$ is as yet unknown but is determined by the functional response of the fish predator to cercarial density.

The immigration vector γ, determining the number of immigrant larval parasites entering the adult parasite population on a single fish will thus be of the form,

$$\gamma(t) = \begin{pmatrix} \gamma_*(C_t) \\ 0 \\ \cdot \\ \cdot \\ \cdot \\ 0 \end{pmatrix}$$

where

$$\gamma_*(C_t) = \sum_{t=0}^{m} g_t C_t(t)$$

(*m* representing the oldest age class in the cercarial population).

The cercarial population reaches an equilibrium state after approximately 48 h (Fig. 4), a time period which is very short in comparison to the ten week period taken by the adult parasite population to achieve a steady state (Fig. 8). After 48 h the immigration term is thus of the form,

$$\gamma_*(\bar{C}) = \sum_{t=0}^{\dot{m}} g_t \bar{C}_t$$

where $\bar{C}_t$ is the equilibrium population size of the number of cercariae in age class *t*. The total number of immigrants entering the adult parasite population per unit period of time is thus a constant once the cercarial population is at equilibrium.

It therefore becomes apparent, that but for the very early time periods, the framework of the model represented by equation (15) is appropriate to describe the dynamics of an adult parasite population in an experimental chamber containing a constant number of fish and infected snails. However, before precise predictions of the dynamics of such parasite populations can be made, further experimental work is required to determine the nature of the predation losses of the larval parasite population due to the final fish host.

DISCUSSION

The experimental investigations described in this paper, of the population processes influencing the cercarial and adult populations of *Transversotrema patialense*, illustrate a number of general points concerning the dynamics of digenean parasites.

The survival characteristics of both cercarial and adult parasite populations were shown to be age dependent, where the death rate increased as the parasites aged. Although the biological causes for such patterns differ widely, as illustrated by the two developmental stages of *T. patialense*, the exponential form of the instantaneous death rate appears to be very common among helminth parasites (Anderson 1976b). The maximum life span of larval parasite stages such as the cercariae and miracidia of digeneans (Oliver & Short 1956) which are in general thought to be non-feeding and thus possess a finite energy reserve, are invariably short, the duration being of the order of hours rather than days. It is these stages which are responsible for the transmission of the parasite from one host to the next in the life cycle and thus maximum survival in the early stages of the life span is important since, as illustrated by the cercariae of *T. patialense*, infectivity which may itself be age dependent is often at a peak during this period. In general adult worm life spans, however, are usually much longer in comparison, since

it is these stages which are responsible for egg production, the magnitude of which determines the transmission rate of the parasite from final to intermediate host (Fig. 1). Egg production itself is often age dependent (Anderson 1976b) and thus an optimum strategy for the parasite would entail maximum survival during the age classes when peak egg production occurs. Little evidence is available at present to examine this possibility, but other factors such as host generated immune responses which often influences both survival (Jarrett *et al.* 1968) and egg production (Michel 1967) will also play a major role in determining observed patterns.

A surprising feature of cercarial production was the remarkable temporal constancy in daily output of infected snails. The apparent randomness of the time sequences of output from individual snails is also of interest. In terms of the dynamics of natural populations of parasites and hosts it is apparent that in order to predict the total daily cercarial output into an aquatic habitat, a knowledge of the number of snails in each size (age) class of hosts is ideally required since there are indications that individual cercarial production is linked to the size of the host. For the purposes of experimental work, however, the introduction of a known number of infected snails into an experimental tank will result in a constant cercarial input into the system, provided all the infected snails survive the duration of the experiment. The most important factor determining output appears to be the nutritional state of the molluscs, starvation resulting in the cessation of cercarial production. In natural habitats, periods of adverse climatic conditions, due to seasonal trends, could thus lead to marked seasonality in cercarial production and thus in the dynamics of the complete parasite life cycle. It was interesting to note, however, that the larval stages of the parasite were capable of resuming their production once conditions improved, the food supply to the snail being restarted.

Of the population processess examined in this present study one of the most important is the infection of the fish host by the cercariae. Infectivity itself appears to be a relatively simple process. Although dependent on the age of the larvae, the rate was shown to be simply proportional to the density of cercariae in the habitat and essentially involved chance contacts between host and parasite. Of more importance, due to its influence on the cercarial density and thus the rate of infection, is the predatory activity of the fish host. The form of the functional response of the fish host to cercarial density undoubtedly plays a major role in determining the dynamics of the complete parasite life cycle. Preliminary observations on *Brachydanio rerio* conform to the generally accepted premise that relatively sophisticated predators such as fish exhibit type III functional responses. The response curve is often sigmoid approaching an upper asymptote (Holling 1965), the fish learning to spend an increasing proportion of its time feeding on the prey as density increases. These sorts of functional responses have recently been discussed in detail with respect to arthropod predators and parasitoids (Hassell *et al.* 1976, 1977). These authors have made the important observation, also noted by other workers, in particular Holling (1965) and Murdoch & Oaten (1975), that type III responses can potentially stabilize the interaction between predator and prey populations. Such influences may occur in the case of the cercariae of *Transversotrema patialense* and the fish predator. If the equilibrium cercarial population size is in the region where the rate of predation is an increasing function of prey density, then the cercariae death rate will be density dependent, high densities resulting in an increase in the rate. In such a case, the predatory activity of the final fish host may play a major role in regulating the interaction between host and parasite. The exact form of the functional relationship between the predatory activity of *Brachydanio rerio* and the density of cercariae of

Transversotrema patialense, plus the influence of this process on the infection of the final host will be considered in greater detail in a further publication.

The population models described in this paper are constructed around the framework of immigration-death processes and thus predict growth to a single stable equilibrium state. This pattern of behaviour is artificial, being caused by the assumption of constant immigration rates and the examination of compartments of the life cycle in isolation (Fig. 1). The behaviour of one compartment or population is in reality determined to a large extent by the behaviour of the preceding compartment in the cycle. The dynamics of the complete life cycle can thus only be fully understood by regarding all the interconnecting compartments as a single unit possessing its own unique dynamical behaviour.

The complex nature of many helminth parasite life cycles has tended to lead to confusion in the parasitological literature concerning the existence and relevance of density dependent regulatory mechanisms. It has been argued that the complexity of the cycles themselves, resulting from the many host and parasite populations involved (Fig. 1) generates the stability of the system. It is difficult at present to assess whether the complexity of parasite life cycles enhances or decreases the stability of such systems. The lack of knowledge in this area, however, does not detract from the obvious need for the presence of density-dependent processes in order to regulate population growth in complex parasite life cycles. The numerous host and distinct parasite populations involved in digenean life cycles leads to the presence of large numbers of rate parameters which creates many opportunities for density-dependent responses to occur.

In the case of *T. patialense*, the probable existence of two such processes, the predatory activities of the fish host and the influence of multiple miracidial infections on the snail host, have already been suggested. It is highly likely, however, that other such processes occur. The survival and rate of egg production of the adult parasite on the fish host are obvious examples due to the finite nature of resources such as number of scale recesses and available food on the surface of a fish. Preliminary observations also suggest that both the survival and reproductive capabilities of the snail intermediate host *Melanoides tuberculata*, are adversely affected by infection with *Transversotrema patialense*. Parasite induced alterations in host mortality and reproduction have been recorded for infections of other larval digeneans (Pan 1965). Finally, very heavy infections of the fish host with the adult parasites may also lead to increased host mortality.

It thus appears likely that the life cycle of *T. patialense* contains many density dependent population processes. The dynamical behaviour of the system will thus be complex and it is possible that a series of stable equilibrium states exist for both the host and parasite populations in a given habitat.

Density independent factors, such as water temperature will also play a major role in determining the dynamics of a parasite which utilises two poikilothermic hosts and has two different larval stages free-living in the aquatic habitat. In the experiments described in this paper, temperature was maintained at a constant level, but in natural habitats seasonal fluctuations in this factor will no doubt lead to cyclic oscillations in population size (Anderson 1976a).

To conclude, it is very apparent from the preceding discussion that a great deal of experimental work remains to be done before even a superficial understanding of the dynamics of this complex digenean life cycle can be achieved. The many population processes, outlined in Fig. 1 all require detailed experimental attention. Due to the manipulability of the *T. patialense* life cycle in the laboratory, we feel optimistic about attaining some degree of insight into the dynamical properties of this particular host-

helminth parasite system. We feel that it is important to gain an understanding of such population interactions due to the economic importance of many parasitic species with similar complex life cycles.

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SUMMARY

(1) The biological components of the population dynamics of the cercarial and adult stages of the ectoparasitic digenean, *Transversotrema patialense* are examined within an experimental framework where temperature and dark-light regimes remained constant.

(2) The survival characteristics of the larval and adult parasites are shown to be age-dependent. At 24° C the maximum life span of the cercariae is approximately 44 h while that of the adult is close to 10 weeks.

(3) The factors influencing cercarial production by the molluscan intermediate host *Melanoides tuberculata* are discussed. The size of the snails and their nutritional status are suggested to be important determinants of larval parasite production.

(4) The temporal constancy of cercarial output by snails of unknown infection origin is discussed in relation to single and multiple miracidial infections. It is suggested that multiple infections do not lead to greatly increased larval production.

(5) Losses from the free-living cercarial populations are shown to be due to three processes; natural mortalities, infection of the final fish host and predation by the fish.

(6) Infection of the fish host, *Brachydanio rerio*, is shown to be essentially a chance process depending on random contacts between host and cercariae. The rate of infection is thus directly proportional to the density of larvae in an aquatic habitat, although the degree of proportionality is shown to be dependent on the age of the cercariae.

(7) It is suggested that predation is a very important component of the dynamics of the larval parasite population and that the functional response of the fish predator to prey density is sigmoid in form.

(8) The survival characteristics of the adult parasite on the fish host are shown to remain constant in form, in parasite populations subject to temporally overlapping infections. This observation is taken to suggest that host generated immunological mechanisms are not important in the dynamics at the parasite population levels used in the experiments.

(9) The regulatory influences in the complete life cycle of *Transversotrema patialense* are discussed in general terms. The existence of a number of density dependent processes in the life cycle is suggested, including predation, infection of the snail host, adult parasite survival and fecundity and mortality of infected intermediate and final hosts.

(10) Deterministic immigration-death models, incorporating population age structure are developed to describe the dynamics of both cercarial and adult parasite populations. Stochastic influences are discussed in relation to immigration and mortality.

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